Supplementary material

Effect of polar headgroup and spacer length on DNA transfection of cholesterol-based cationic lipids

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NMR data of all synthesized compounds

3β-[*N*-(2-aminoethyl)carbamoyl]cholesterol (8): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.97 (3H, s, H-19-Chol), 0.89-2.40 (30H, m, protons in cholesteryl skeleton), 2.79 (2H, br q, $H_2NC\underline{H}_2CH_2NH(CO)O$), 3.20 (2H, t, $H_2NCH_2C\underline{H}_2NH(CO)O$), 4.46 (1H, br s, H-3-Chol), 5.04 (1H, t, $H_2N(CH_2)_2N\underline{H}(CO)O$), 5.34 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.2, 28.0, 28.1, 28.2, 31.8, 35.7, 36.1, 36.5, 36.9, 39.5, 39.7, 41.7, 42.3, 49.9, 56.1, 56.6, 74.3, 122.4, 139.8 (carbons in cholesteryl skeleton), 38.5, 43.2 ($NH_2\underline{C}H_2\underline{C}H_2NHCO_2$-Chol), 156.4 ($\underline{C}O_2$-Chol).

3β-[*N*-(3-aminopropyl)carbamoyl]cholesterol (9): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18-Chol), 0.82 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.87 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.97 (3H, s, H-19-Chol), 0.97-2.40 (30H, m, protons in cholesteryl skeleton), 2.74 (2H, t, $H_2NCH_2(CH_2)_2NH(CO)O$), 3.22 (2H, br t, $H_2N(CH_2)_2CH_2NH(CO)O$), 3.43 (1H, s, $H_2N(CH_2)_3NH(CO)O$), 4.44 (1H, br s, H-3-Chol), 5.07 (1H, br s, $H_2N(CH_2)_2NH(CO)O$), 5.33 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.2, 28.0, 28.1, 28.2, 31.6, 31.8, 35.8, 36.1, 36.5, 36.9, 39.5, 39.7, 42.2, 49.9, 56.1, 56.6, 74.2, 122.4, 139.8 (carbons in cholesteryl skeleton), 38.9, 38.5, 31.8 ($NH_2(CH_2)_3NHCO_2$-Chol), 156.5 (CO_2-Chol).

3β-[*N*-(2-(*N'*-(Fmoc-lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (10): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18-Chol), 0.84 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.91 (3H, s, H-19-Chol), 0.92-2.40 (35H, m, Protons in cholesteryl skeleton and Fmoc H-9"), 1.45 (9H, s, $O(C\underline{H}_3)_3$), 3.07 (2H, br s, $HNCH(CH_2)_3C\underline{H}_2NH$), 3.27 (2H, br s, $HNCH_2C\underline{H}_2NH(CO)O$), 3.34 (2H, br s, $HNC\underline{H}_2CH_2NH(CO)O$), 4.10 (1H, br s, H-3-Chol), 4.17 (1H, t, $HNC\underline{H}(CH_2)_4NH$), 4.35 (2H, d, $CH\text{-}C\underline{H}_2O(CO)$), 4.41 (1H, br s, $HN(CH_2)_2N\underline{H}(CO)O$), 4.69 (1H, br s, $HNCH(CH_2)_4N\underline{H}$), 5.28 (1H, br s, H-6-Chol), 5.67 (1H, br s, $\underline{H}N(CH_2)_2NH(CO)O$), 6.87 (1H, s, $\underline{H}NCH(CH_2)_4NH$), 7.27 (2H, t, Fmoc H-3", Fmoc H-6"), 7.36 (2H, t, Fmoc H-2", Fmoc H-7"), 7.56 (2H, d, Fmoc H-4", Fmoc H-5"), 7.73 (2H, d, Fmoc H-1", Fmoc H-8"); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.9, 19.2, 21.0, 22.4, 22.5, 23.8, 24.2, 28.0, 28.2, 29.6, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.5, 39.5, 39.7, 42.3, 49.9, 56.1, 56.6, 74.6, 122.5, 139.6 (carbons in cholesteryl skeleton), 28.4 ($CO_2C(\underline{C}H_3)_3$), 38.5, 39.9 ($NH\underline{C}H_2\underline{C}H_2NHCO_2$-Chol), 22.8, 31.8, 32.0, 40.5, 54.9 ($NH\underline{C}H(\underline{C}H_2)_4NHBoc$), 79.3 ($CO_2\underline{C}(CH_3)_3$), 47.1, 67.1, 119.9, 125.1, 127.1, 127.7, 141.3, 143.8, 157.0 (carbons in Fmoc structure), 156.3 ($\underline{C}O_2C(CH_3)_3$), 156.3 ($\underline{C}O$-Chol), 172.4 ($\underline{C}O$ amide).

3β-[*N*-(3-(*N′*-(Fmoc-lysin(Boc)amide))aminopropyl)carbamoyl]cholesterol (11): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18-Chol), 0.84 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.95 (3H, s, H-19-Chol), 0.95-2.40 (37H, m, protons in cholesteryl skeleton and H-9"), 1.40 (9H, s, $O(C\underline{H}_3)_3$), 3.08 (2H, br s, $HNCH(CH_2)_3C\underline{H}_2NH$), 3.16 (2H, br s, $HN(CH_2)_2C\underline{H}_2NH(CO)O$), 3.28 (2H, s, $HNC\underline{H}_2(CH_2)_2NH(CO)O$), 4.11 (1H, br s, H-3-Chol), 4.18 (1H, t, $HNC\underline{H}(CH_2)_4NH$), 4.36 (2H, m, $CHC\underline{H}_2O(CO)$), 4.65 (1H, s, $NHCH(CH_2)_4N\underline{H}$), 5.08 (1H, s, $HN(CH_2)_3N\underline{H}(CO)O$), 5.31 (1H, br s, H-6-Chol), 5.58 (1H, s, $\underline{H}N(CH_2)_3NH(CO)O$), 6.84 (1H, s, $\underline{H}NCH(CH_2)_4NH$), 7.28 (2H, t, CH-3", CH-6"), 7.37 (2H, t, CH-2", CH-7"), 7.57 (2H, d, CH-4", CH-5"), 7.73 (2H, d, CH-1", CH-8"); ^{13}C NMR (100 MHz, $CDCl_3$): 11.9, 18.7, 19.3, 21.0, 22.5, 22.6, 23.8, 24.3, 28.0, 28.1, 29.6, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.5, 39.5, 39.7, 42.3, 49.9, 56.1, 56.7, 74.5, 122.6, 139.7 (carbons in cholesteryl skeleton), 28.5 ($CO_2C(\underline{C}H_3)_3$), 28.3, 37.3, 39.5 ($NH_2(\underline{C}H_2)_3NHCO_2$-Chol), 22.8, 30.0, 32.2, 40.1, 55.0 ($NH\underline{C}H(\underline{C}H_2)_4NHBoc$), 79.3 ($CO_2\underline{C}(CH_3)_3$), 47.2, 67.1, 120.0, 125.1, 127.1, 127.7, 141.3, 143.8, 156.9 (carbons in Fmoc structure), 156.3 ($\underline{C}O_2C(CH_3)_3$), 156.3 ($\underline{C}O$-Chol), 172.1 (CO amide).

3β-[*N*-(2-(*N′*-(lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (12): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.97 (3H, s, H-19-Chol), 0.97-2.40 (36H, m, protons in cholesteryl skeleton), 1.40 (9H, s, $O(C\underline{H}_3)_3$), 3.08 (2H, br s, $HNCH(CH_2)_3C\underline{H}_2NH$), 3.28 (2H, br s, $HNCH_2C\underline{H}_2NH(CO)O$), 3.34 (3H, br s, $HNC\underline{H}_2CH_2NH(CO)O$ and $H_2NC\underline{H}(CH_2)_4NH$), 4.44 (1H, br s, H-3-Chol), 4.63 (1H, s, $HN(CH_2)_2N\underline{H}(CO)O$), 5.21 (1H, s, $\underline{H}N(CH_2)_2NH(CO)O$), 5.33 (1H, br s, H-6-Chol), 7.62 (1H, br s, $\underline{H}_2NCH(CH_2)_4NH$); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.3, 28.1, 28.2, 29.8, 31.8, 35.8, 36.2, 36.5, 36.9, 38.5, 39.5, 39.7, 42.3, 50.0, 56.1, 56.7, 74.8, 122.5, 139.8 (carbons in cholesteryl skeleton), 28.4 ($CO_2C(\underline{C}H_3)_3$),

39.5, 40.1 (NH<u>C</u>H$_2$<u>C</u>H$_2$NHCO$_2$-Chol), 22.8, 28.0, 34.4, 41.1, 55.0 (NH$_2$<u>C</u>H(<u>C</u>H$_2$)$_4$NHBoc), 79.1 (CO_2<u>C</u>(CH$_3$)$_3$), 156.1 (<u>C</u>O$_2$C(CH$_3$)$_3$), 156.7 (<u>C</u>O$_2$-Chol), 175.8 (<u>C</u>O amide).

3β-[*N*-(3-(*N′*-(lysin(Boc)amide))aminopropyl)carbamoyl]cholesterol (13): ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.64 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.97 (3H, s, H-19-Chol), 0.97-2.40 (37H, m, protons in cholesteryl skeleton), 1.40 (9H, s, O(C<u>H</u>$_3$)$_3$), 3.07 (2H, br s, H$_2$NCH(CH$_2$)$_3$C<u>H</u>$_2$NH), 3.15 (2H, br s, HN(CH$_2$)$_2$C<u>H</u>$_2$NH(CO)O), 3.27 (3H, br s, HNC<u>H</u>$_2$(CH$_2$)$_2$(CO)O and H$_2$NC<u>H</u>(CH$_2$)$_4$NH), 3.59 (1H, s, HN(CH$_2$)$_3$N<u>H</u>(CO)O), 4.43 (1H, br s, H-3-Chol), 4.80 (1H, s, H$_2$NCH(CH$_2$)$_4$N<u>H</u>), 5.33 (1H, br s, H-6-Chol), 5.38 (1H, br s, <u>H</u>N(CH$_2$)$_3$NH(CO)O), 7.85 (1H, s, <u>H</u>$_2$NCH(CH$_2$)$_4$NH); ^{13}C NMR (100 MHz, CDCl$_3$): 11.8, 18.7, 19.3, 21.0, 22.8, 22.5, 23.8, 24.3, 28.0, 28.1, 28.2, 31.8, 31.9, 35.8, 36.2, 36.5, 37.0, 39.5, 39.7, 42.3, 42.3, 50.0, 56.1, 56.7, 74.3, 122.4, 139.9 (carbons in cholesteryl skeleton), 28.4 (CO_2C(<u>C</u>H$_3$)$_3$), 28.4, 37.6, 38.6 (NH$_2$(<u>C</u>H$_2$)$_3$NHCO$_2$-Chol), 22.8, 29.7, 33.1, 40.0, 54.4 (NH$_2$<u>C</u>H(<u>C</u>H$_2$)$_4$NHBoc), 79.1 (CO_2<u>C</u>(CH$_3$)$_3$), 156.2 (<u>C</u>O$_2$C(CH$_3$)$_3$), 156.3 (<u>C</u>O$_2$-Chol), 156.6 (CO amide).

3β-[*N*-(2-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (14): ^{1}H NMR (400 MHz, CDCl$_3$ + 5 drops of CD$_3$OD): δ 0.64 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.96 (3H, s, H-19-Chol), 0.96-2.40 (34H, m, protons in cholesteryl skeleton), 1.40 (9H, s, O(C<u>H</u>$_3$)$_3$), 3.09 (4H, br s, H$_2$NCH(CH$_2$)$_3$C<u>H</u>$_2$NHBoc and HNCH$_2$C<u>H</u>$_2$NH(CO)O), 3.28 (9H, br s, N$^+$(CH$_3$)$_3$), 3.51 (2H, br s, HNC<u>H</u>$_2$CH$_2$NH(CO)O), 4.43 (1H, br s, H-3-Chol), 4.84 (1H, br s, <u>H</u>NCH$_2$CH$_2$NH(CO)O), 4.98 (1H, br s, H$_2$NC<u>H</u>(CH$_2$)$_4$NHBoc), 5.18 (1H, br s, HNCH$_2$CH$_2$N<u>H</u>(CO)O), 5.32 (1H, br s, H-6-Chol), 6.03 (1H, br s, NCH(CH$_2$)$_4$N<u>H</u>); ^{13}C NMR (100 MHz, CDCl$_3$ + 5 drops of CD$_3$OD): 11.7, 18.6, 19.2, 20.9, 22.4, 22.7, 23.7, 24.2, 27.9, 28.0,

28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.9, 39.4, 39.6, 42.2, 49.9, 56.0, 56.6, 73.5, 122.4, 139.7 (carbons in cholesteryl skeleton), 28.3 ($CO_2C(\underline{C}H_3)_3$), 38.4, 39.6 ($NH(\underline{C}H_2)_2NHCO_2$-Chol), 22.3, 29.6, 36.4, 39.8, 74.3 ($((CH_3)_3N^+\underline{C}H(\underline{C}H_2)_4NHBoc$), 52.4 ($((\underline{C}H_3)_3N^+$), 79.2 ($CO_2\underline{C}(CH_3)_3$), 156.5 ($\underline{C}O_2C(CH_3)_3$), 156.6 ($\underline{C}O_2$-Chol), 172.6 (CO amide).

3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysin(Boc)amide)) aminopropyl)carbamoyl]cholesterol (15): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (3H, s, H-18-Chol), 0.80 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.85 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.94 (3H, s, H-19-Chol), 1.01-2.25 (34H, m, protons in cholesteryl skeleton), 1.36 (9H, s, $CO_2(C\underline{H}_3)_3$), 1.05 (4H, m, $NHCHCH_2(C\underline{H}_2)_2CH_2NHBoc$), 1.50 (2H, m, $NHCH_2C\underline{H}_2CH_2NHCO_2$-Chol), 1.79 (2H, m, $NHCHC\underline{H}_2(CH_2)_3NHBoc$), 3.00 (2H, m, $NH(CH_2)_2C\underline{H}_2NHCO_2$-Chol), 3.12 (2H, m, $(NHCH(CH_2)_3)C\underline{H}_2NHBoc$), 3.18 (9H, s, $(C\underline{H}_3)_3N^+$), 3.27 (2H, m, $NHC\underline{H}_2(CH_2)_2NHCO_2$-Chol), 3.33 (1H, br m, $NHC\underline{H}(CH_2)_4NHBoc$), 4.38 (1H, br s, H-3-Chol), 5.29 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, CDCl3): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.3, 28.0, 28.1, 28.2, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 39.5, 39.7, 40.5, 42.3, 50.0, 56.1, 56.7, 74.7, 122.6, 139.7 (carbons in cholesteryl skeleton), 28.4 ($CO_2C(\underline{C}H_3)_3$), 28.4, 38.5, 39.8 ($NH(\underline{C}H_2)_3NHCO_2$-Chol), 23.2, 29.6, 31.7, 40.4, 77.2 ($((CH_3)_3N^+\underline{C}H(\underline{C}H_2)_4NHBoc$), 53.2 ($((\underline{C}H_3)_3N^+$), 79.2 ($CO_2\underline{C}(CH_3)_3$), 156.3 ($\underline{C}O_2C(CH_3)_3$), 157.0 ($\underline{C}O_2$-Chol), 172.3 (CO amide).

3β-[*N*-(2-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide)))) aminoethyl)carbamoyl]cholesterol (16): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.96 (3H, s, H-19-Chol), 0.96-2.40 (32H, m, protons in cholesteryl skeleton), 1.45 (27H, s, $O(C\underline{H}_3)_3$), 3.07 (2H, br s, $HNCH(CH_2)_3C\underline{H}_2NH$), 3.26 (2H, br s, $HNCH_2C\underline{H}_2NH(CO)O$), 3.29 (1H, br s, $HNC\underline{H}(CH_2)_4NH$), 3.39 (2H, br s, $HNC\underline{H}_2CH_2NH(CO)O$), 4.10 (2H, q, $HNCHC\underline{H}_2(CH_2)_3NH$),

4.38 (1H, br s, $HNCH_2CH_2N\underline{H}(CO)O$), 4.40 (1H, br s, $\underline{H}NCH_2CH_2NH(CO)O$), 4.41 (1H, br s, H-3-Chol), 4.62 (1H, s, $HNCH(CH_2)_4N\underline{H}$), 5.27 (1H, br s, $\underline{H}NCH(CH_2)_4NH$), 5.29 (1H, br s, $\underline{H}N(C=N)NH$), 5.33 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.7, 19.3, 21.0, 22.5, 22.8, 23.8, 24.3, 28.0, 28.1, 28.2, 31.2, 31.8, 35.8, 36.2, 36.5, 36.9, 38.5, 39.5, 39.7, 42.3, 50.0, 56.1, 56.7, 74.4, 122.5, 139.8 (carbons in cholesteryl skeleton), 28.0, 28.2, 28.4 ($3\times CO_2C(\underline{C}H_3)_3$), 38.5, 40.1 ($NH\underline{C}H_2\underline{C}H_2NHCO_2$-Chol), 23.8, 29.6, 31.9, 39.9, 54.5 ($NH\underline{C}H(\underline{C}H_2)_4NHBoc$), 79.4, 80.7, 84.1 ($3\times CO_2\underline{C}(CH_3)_3$), 152.6, 156.5, 156.6 ($3\times\underline{C}O_2C(CH_3)_3$), 156.0 ($NH(\underline{C}$=NBoc)), 156.0 ($\underline{C}O_2$-Chol), 171.2 (CO amide).

3β-[*N*-(3-(*N'*-(*N''*-(*N'''*,*N'''*-diBoc(guanidinyl-lysin(Boc)amide))))aminopropyl)carbamoyl]cholesterol (17): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18-Chol), 0.82 (6H, d, J = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, J = 6.4 Hz, H-21-Chol), 0.98 (3H, s, H-19-Chol), 1.05-2.33 (32H, m, protons in cholesteryl skeleton), 1.40, 1.45, 1.47 (27H, s, $3\times CO_2(C\underline{H}_3)_3$), 1.62 (2H, m, $HNCH(CH_2)_2C\underline{H}_2CH_2NHBoc$), 1.83 (4H, m, $HNCH(C\underline{H}_2)_2(CH_2)_2NHBoc$), 1.99 (2H, m, $NHCH_2C\underline{H}_2CH_2NHCO_2$-Chol), 3.08-3.28 (6H, m, ($NHC\underline{H}_2CH_2C\underline{H}_2NHCO_2$-Chol) and ($NHCH(CH_2)_3C\underline{H}_2NHBoc$)), 4.44 (1H, br s, H-3-Chol), 4.50 (1H, s, $HNC\underline{H}(CH_2)_4NHBoc$), 5.34 (1H, br s, H-6-Chol), 4.61, 5.15, 7.14, 8.74, 11.32 (5H, s 5×NH); ^{13}C NMR (100 MHz, $CDCl_3$): 11.9, 18.7, 19.4, 21.0, 22.6, 22.6, 23.8, 24.3, 28.0, 28.3, 29.8, 31.4, 31.9, 35.8, 36.2, 36.6, 37.0, 38.6, 39.5, 39.7, 40.2, 50.0, 56.1, 56.7, 74.3, 122.4, 139.9 (carbons in cholesteryl skeleton), 28.1, 28.2, 28.4 ($3\times CO_2C(\underline{C}H_3)_3$), 29.6, 37.4, 38.6 ($NH(\underline{C}H_2)_3NHCO_2$-Chol), 22.8, 31.3, 31.9, 42.1, 54.5 ($NH\underline{C}H(\underline{C}H_2)_4NHBoc$), 79.4, 79.1, 84.0 ($3\times CO_2\underline{C}(CH_3)_3$), 155.4, 156.0, 156.6 ($3\times\underline{C}O_2C(CH_3)_3$), 156.0 ($NH(\underline{C}$=NBoc)), 156.0 ($\underline{C}O_2$-Chol), 171.2 (CO amide).

3β-[*N*-(2-(*N'*-lysinamide)aminoethyl)carbamoyl]cholesterol (1A): ^{1}H NMR (400 MHz, $CDCl_3$ + 10 drops of CD_3OD): δ 0.53 (3H, s, H-18-Chol), 0.72 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.77 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.86 (3H, s, H-19-Chol), 0.86-2.40 (42H, m, protons in cholesteryl skeleton), 2.77 (2H, t, $HNCH_2C\underline{H}_2NH(CO)O$), 3.09 (2H, m, $H_2NCH(CH_2)_3C\underline{H}_2NH_2$), 3.22 (2H, br t, $HNC\underline{H}_2CH_2NH(CO)O$), 3.67 (1H, br t, $H_2NC\underline{H}(CH_2)_4NH_2$), 4.27 (1H, br s, H-3-Chol), 5.21 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$ + 10 drops of CD_3OD): 11.5, 18.4, 19.0, 21.5, 22.2, 22.5, 23.6, 24.0, 27.7, 28.0, 30.3, 31.6, 35.6, 35.9, 36.3, 36.7, 38.2, 39.5, 39.7, 42.1, 49.8, 55.9, 56.5, 74.5, 122.3, 139.5 (carbons in cholesteryl skeleton), 38.7, 39.5 ($NH\underline{C}H_2\underline{C}H_2NHCO_2$-Chol), 26.0, 30.0, 34.4, 39.3, 52.6 ($NH_2\underline{C}H(\underline{C}H_2)_4NH_2$), 157.0 ($\underline{C}O_2$-Chol), 169.1 (CO amide).

3β-[*N*-(3-(*N'*-lysinamide)aminopropyl)carbamoyl]cholesterol (4A): ^{1}H NMR (400 MHz, $CDCl_3$ + 10 drops of CD_3OD): δ 0.60 (3H, s, H-18-Chol), 0.78 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.84 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.93 (3H, s, H-19-Chol), 0.93-2.40 (43H, m, protons in cholesteryl skeleton), 2.62 (2H, t, $HNC\underline{H}_2(CH_2)_2NH(CO)O$), 3.08 (2H, br s, $HN(CH_2)_2C\underline{H}_2NH(CO)O$), 3.20 (3H, br m, $H_2NC\underline{H}(CH_2)_4NH_2$ and $H_2NCH(CH_2)_3C\underline{H}_2NH_2$), 4.38 (1H, br s, H-3-Chol), 5.24 (1H, br s, H-6-Chol), 5.56 (1H, br s, $\underline{H}N(CH_2)_3NH(CO)O$); ^{13}C NMR (100 MHz, $CDCl_3$ + 10 drops of CD_3OD): 11.9, 18.7, 19.4, 21.0, 22.6, 22.9, 23.9, 24.3, 28.0, 28.2, 28.3, 31.8, 35.8, 36.2, 36.6, 37.0, 39.5, 39.7, 42.3, 50.0, 56.1, 56.7, 74.3, 122.5, 139.9 (carbons in cholesteryl skeleton), 38.6, 39.5 ($NH(\underline{C}H_2)_3NHCO_2$-Chol), 24.1, 29.7, 35.8, 39.5, 53.5 ($NH_2\underline{C}H(\underline{C}H_2)_4NH_2$).

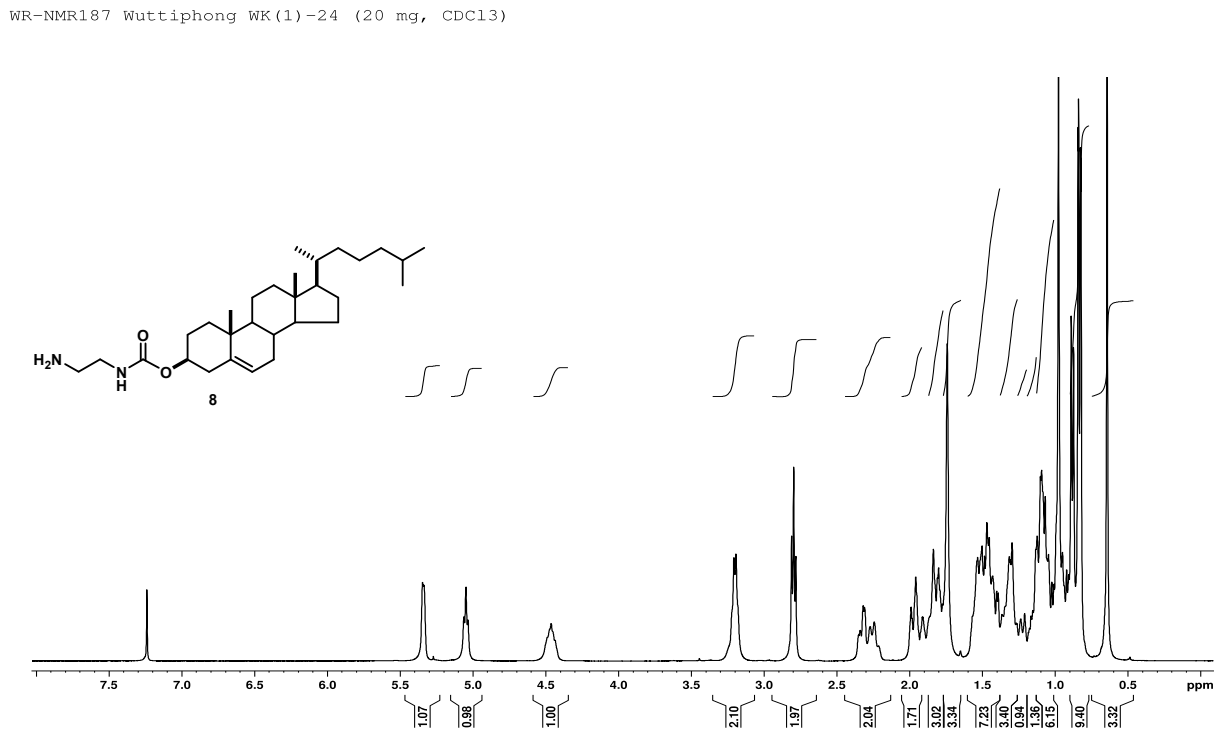
3β-[*N*-(2-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminoethyl)carbamoyl]cholesterol (2A): ^{1}H NMR (400 MHz, $CDCl_3$ + 10 drops of CD_3OD): δ 0.62 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.86 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.94 (3H, s, H-19-Chol), 0.94-2.40 (36H, m, protons in cholesteryl skeleton), 3.18 (9H, br s, $N^+(CH_3)_3$), 3.36 (2H, br s, $NCH(CH_2)_3C\underline{H}_2NH_2$), 3.52 (2H, br s, $HNCH_2C\underline{H}_2NH(CO)O$), 4.27 (2H, br s, $HNC\underline{H}_2CH_2NH(CO)O$), 4.37 (1H, br s, H-3-Chol), 4.47 (1H, br s, $\underline{H}NCH_2CH_2NH(CO)O$), 4.57 (1H, br s, $HNCH_2CH_2N\underline{H}(CO)O$), 5.30 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): 11.8, 18.8, 19.3, 21.0, 22.6, 22.8, 23.9, 24.3, 28.0, 29.7, 31.8, 35.8, 36.2, 36.5, 39.7, 39.4, 42.3, 43.1, 49.9, 56.2, 56.7, 74.1, 122.5, 139.8 (carbons in cholesteryl skeleton), 38.4, 39.0 ($NH\underline{C}H_2\underline{C}H_2CO_2$-Chol), 52.2 ($(\underline{C}H_3)_3N^+$), 29.7, 38.5, 39.5, 74.5 ($(CH_3)_3N^+\underline{C}H(\underline{C}H_2)_4NH_2$), 156.6 ($\underline{C}O_2$-Chol), 166.3 (CO amide).

3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminopropyl)carbamoyl]cholesterol (5A): ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (3H, s, H-18-Chol), 0.77 (6H, d, *J* = 6.4 Hz, H-26, H-27-Chol), 0.83 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.92 (3H, s, H-19-Chol), 1.04-2.50 (34H, m, protons in cholesteryl skeleton), 1.65 (m, 2H, $NHCH_2C\underline{H}_2CH_2NHCO_2$-Chol), 1.91 (m, 4H, $NCHCH_2(C\underline{H}_2)_2CH_2NH_2$), 2.80 (m, 2H, $NCHC\underline{H}_2(CH_2)_3NH_2$) 3.07 (m, 2H, $NHC\underline{H}_2CH_2CH_2NHCO_2$-Chol), 3.14 (9H, br s, $N^+(CH_3)_3$), 3.23 (m, 2H, $NHC\underline{H}_2CH_2CH_2NHCO_2$-Chol), 3.35 (m, 2H, $NCH(CH_2)_3C\underline{H}_2NH_2$), 4.15 (2H, br s, $NC\underline{H}(CH_2)_4NH_2$), 4.46 (1H, br s, H-3-Chol), 5.27 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$ + 5 drops of CD_3OD): 11.7, 18.6, 19.2, 20.9, 22.4, 22.7, 23.7, 24.2, 28.4, 28.8, 29.6, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 39.4, 39.6, 42.2, 43.0, 49.8, 56.0, 56.6, 73.9, 122.4, 139.7 (carbons in cholesteryl skeleton), 27.9, 38.3, 38.9 ($NH(\underline{C}H_2)_3NHCO_2$-Chol), 52.1 ($(CH_3)_3N^+$), 25.4, 29.3, 29.6, 39.3, 74.3 ($(CH_3)_3N^+\underline{C}H(\underline{C}H_2)_4NH_2$), 156.7 ($CO_2$-Chol), 166.0 (CO amide).

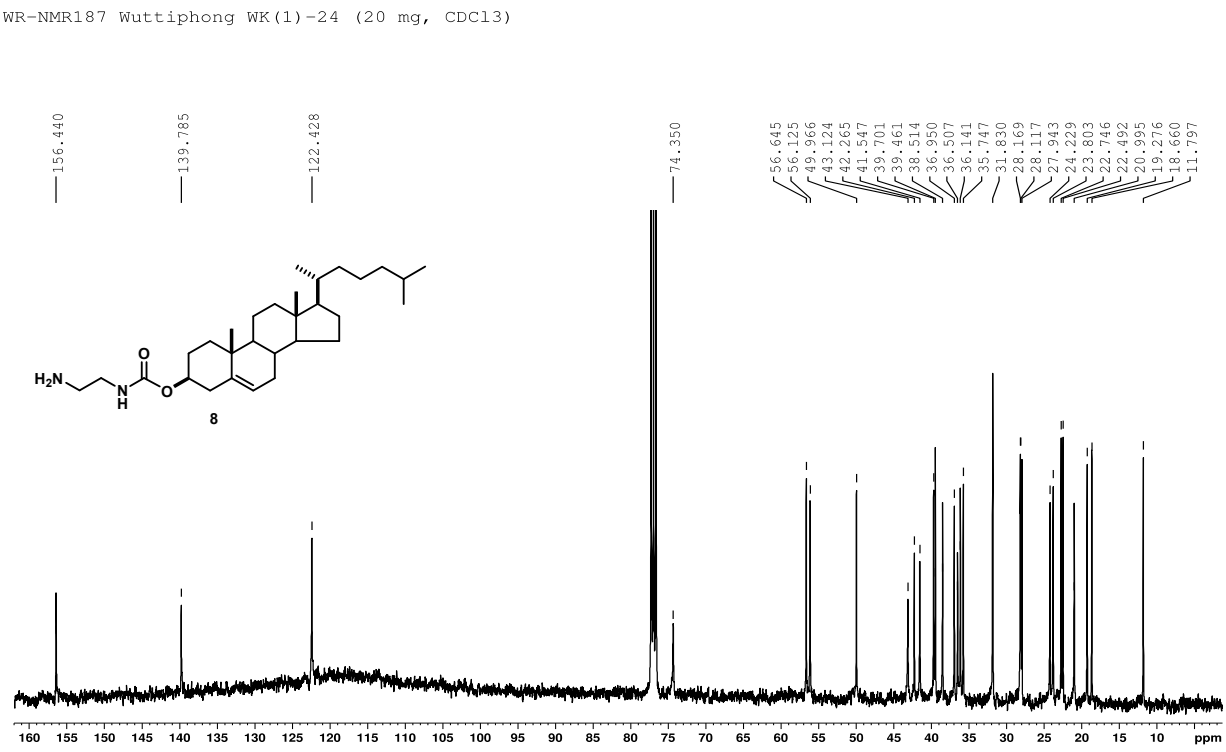
3β-[*N*-(2-(*N′*-(*N″*-guanidinyl-lysinamide))aminoethyl)carbamoyl]cholesterol (3A): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (3H, s, H-18-Chol), 0.83 (6H, d, *J* = 6.5 Hz, H-26, H-27-Chol), 0.88 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.97 (3H, s, H-19-Chol), 0.97-2.50 (41H, m, protons in cholesteryl skeleton), 3.30 (2H, br s, $HNCH(CH_2)_3C\underline{H}_2NH_2$), 4.10 (4H, br s, $HNCH_2C\underline{H}_2NH(CO)O$ and $HNC\underline{H}_2CH_2NH(CO)O$), 4.33 (1H, br s, $HNC\underline{H}(CH_2)_4NH_2$), 4.45 (1H, br s, H-3-Chol), 5.28 (1H, br s, H-6-Chol), 5.59 (1H, $\underline{H}NCH(CH_2)_4NH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): 11.9, 18.7, 19.1, 19.4, 22.6, 22.8, 23.8, 24.0, 28.0, 29.4, 29.7, 30.6, 31.9, 35.7, 36.2, 36.5, 36.8, 38.5, 39.5, 42.3, 44.8, 50.9, 56.2, 56.6, 74.5, 122.5, 139.6 (carbons in cholesteryl skeleton), 38.5, 41.1 ($NH(\underline{C}H_2)_2NHCO_2$-Chol), 22.7, 28.7, 30.6, 41.5, 53.4 ($NH\underline{C}H(\underline{C}H_2)_4NH_2$), 156.2 ($CO_2$-Chol), 161.4 ($NH_2\underline{C}$=N(NHCHCO)), 174.5 (CO amide).

3β-[*N*-(3-(*N′*-(*N″*-guanidinyl-lysinamide))aminopropyl)carbamoyl]cholesterol (6A): ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.59 (3H, s, H-18-Chol), 0.78 (6H, d, *J* = 6.6 Hz, H-26, H-27-Chol), 0.83 (3H, d, *J* = 6.4 Hz, H-21-Chol), 0.91 (3H, s, H-19-Chol), 0.99-2.26 (34H, m, protons in cholesteryl skeleton), 1.43 (m, 4H, $NHCHCH_2C\underline{H}_2C\underline{H}_2CH_2NH_2$), 1.75 (m, 2H, $NCHC\underline{H}_2(CH_2)_3NH_2$), 1.91 (m, 2H, $NHCH_2C\underline{H}_2CH_2NHCO_2$-Chol), 3.30 (m, 4H, ($NHCH(CH_2)_3C\underline{H}_2NH_2$) and ($NHC\underline{H}_2(CH_2)_2NHCO_2$-Chol)), 3.32 (m, 2H, $NH(CH_2)_2C\underline{H}_2NHCO_2$-Chol), 4.34 (1H, br s, H-3-Chol), 5.23 (1H, br s, H-6-Chol); ^{13}C NMR (100 MHz, $CDCl_3$ + 5 drops of CD_3OD): 11.7, 18.5, 19.1, 20.9, 22.4, 22.7, 23.7, 24.1, 27.9, 28.0, 28.1, 31.68, 31.73, 35.7, 36.0, 36.4, 36.8, 39.3, 39.6, 42.1, 49.9, 55.9, 56.5, 74.3, 122.4, 139.6 (carbons in cholesteryl skeleton), 29.2, 38.4 ($NH(\underline{C}H_2)_3NHCO_2$-Chol), 23.7, 27.9, 31.7, 39.4, 53.3 ($NH\underline{C}H(\underline{C}H_2)_4NH_2$), 156.8 ($\underline{C}O_2$-Chol), 159.3 ($NH_2\underline{C}$=NH(NHCHCO)), 175.9 (CO amide).

^{1}H, ^{13}C NMR and HR-ESI-MS Spectra of All Synthesized Compounds



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-aminoethyl)carbamoyl]cholesterol (**8**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-aminoethyl)carbamoyl]cholesterol (**8**)

Mass Spectrum SmartFormula Report

Analysis Info

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Comment			

Acquisition Parameter

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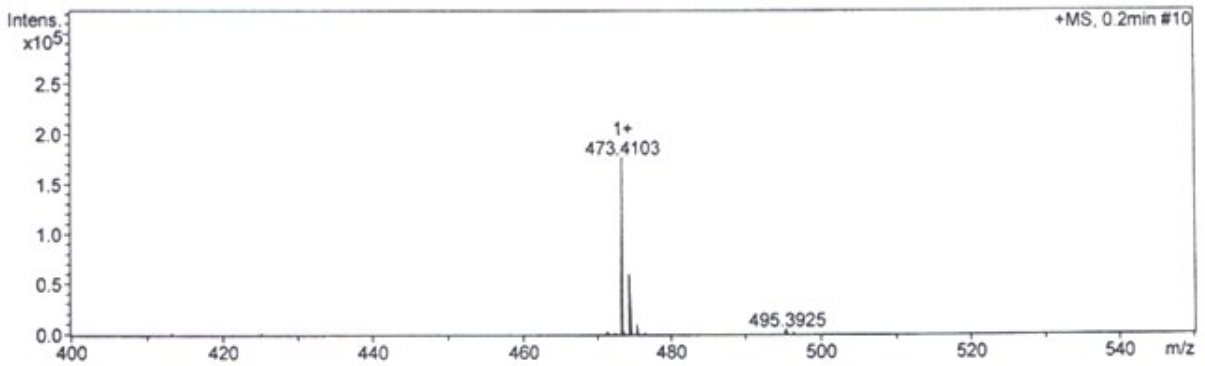
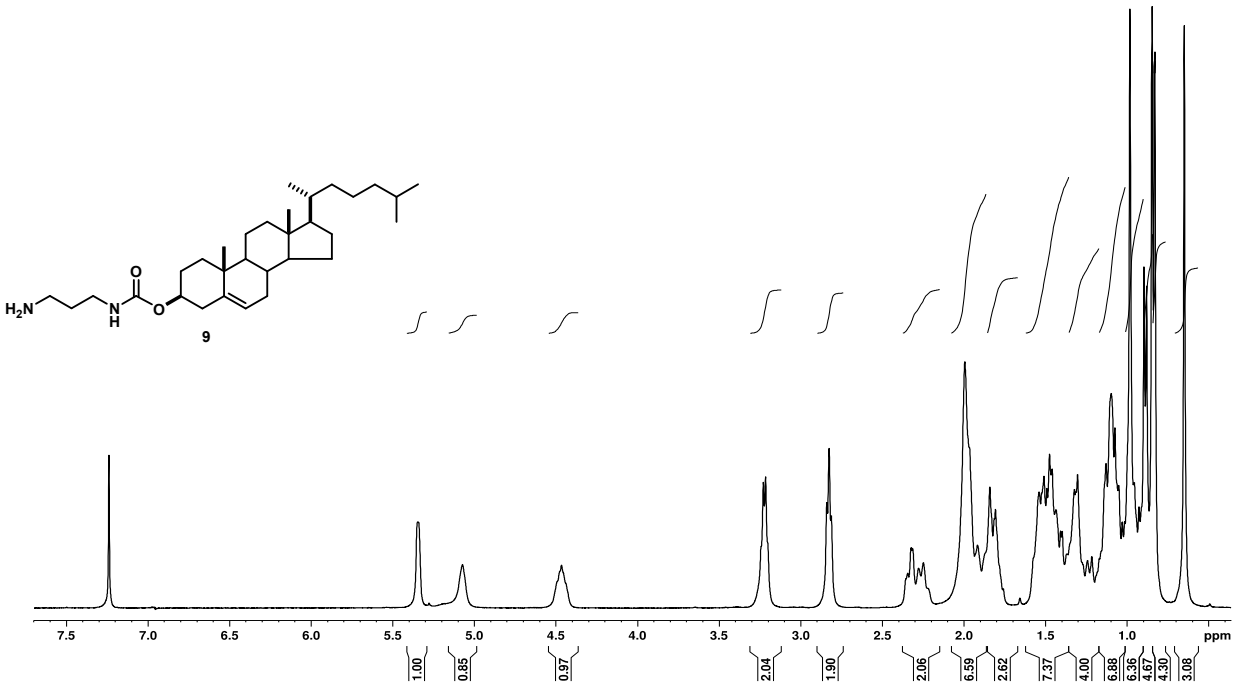
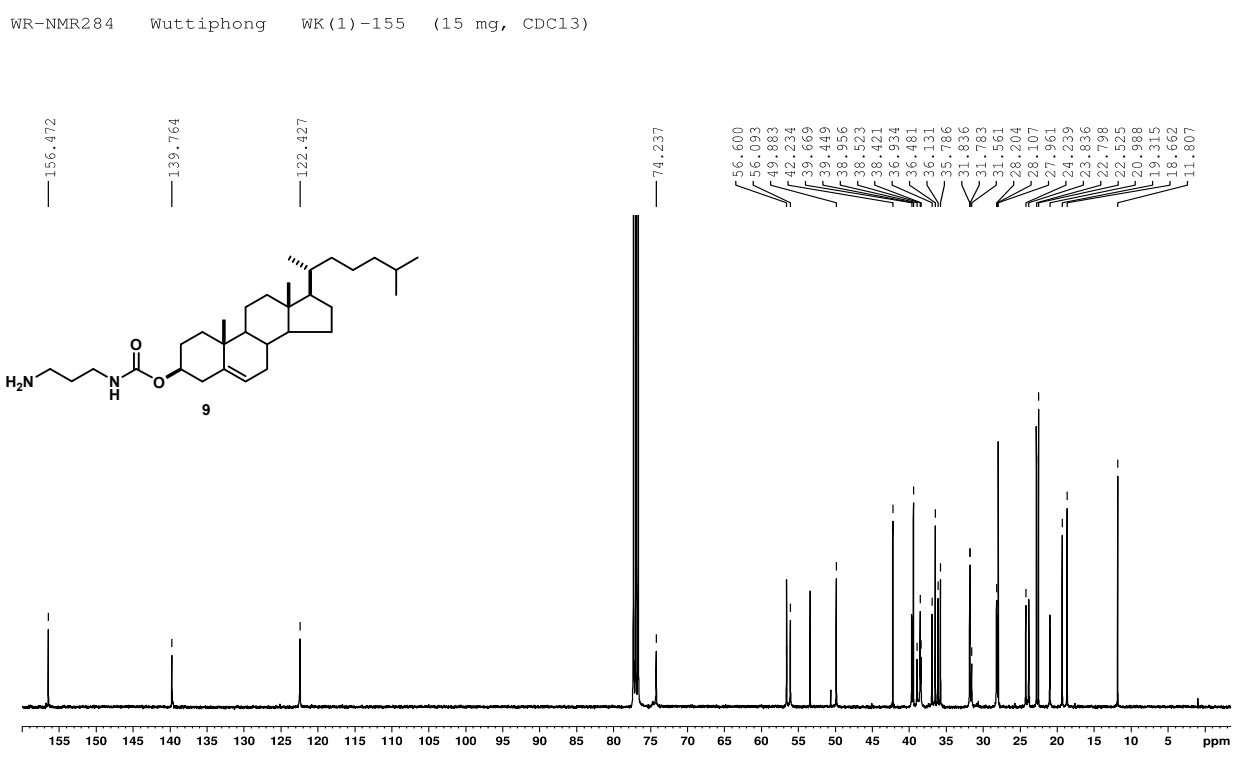


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
369.351742	1	C27H45	369.351578	0.4	-0.1	5.5	ok	even	7.7	13.0	0.3	4.0	0.6	842.7
	1	C27H45	369.351578	0.4	-0.1	5.5	ok	even	7.7	13.0	0.3	4.0	0.6	842.7
	1	C25H46Na	369.349172	-7.0	-6.6	2.5	ok	even	4.4	7.4	2.5	3.5	0.5	842.7
473.410269	1	C30H53N2O2	473.410155	-0.2	-0.1	5.5	ok	even	2.1	3.2	0.3	1.1	0.7	842.7
	2	C35H53	473.414178	8.3	8.5	9.5	ok	even	21.9	33.8	4.0	10.6	0.9	1000.0
	1	C30H53N2O2	473.410155	-0.2	-0.1	5.5	ok	even	2.1	3.2	0.3	1.1	0.7	842.7
	2	C35H53	473.414178	8.3	8.5	9.5	ok	even	21.9	33.8	4.0	10.6	0.9	1000.0
	1	C33H54Na	473.411772	-3.2	3.4	6.5	ok	even	10.5	16.0	1.6	5.0	0.9	842.7
	2	C28H54N2NaO2	473.407750	5.3	-5.2	2.5	ok	even	13.2	21.9	2.5	6.8	0.7	909.6
	1	C30H53N2O2	473.410155	-0.2	-0.1	5.5	ok	even	2.1	3.2	0.3	1.1	0.7	842.7
	1	C28H54N2NaO2	473.407750	5.3	-5.2	2.5	ok	even	13.2	21.9	2.5	6.8	0.7	909.6

HR-ESI-MS Spectrum of 3β-[*N*-(2-aminoethyl)carbamoyl]cholesterol (**8**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-aminopropyl)carbamoyl]cholesterol (**9**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-aminopropyl)carbamoyl]cholesterol (**9**)

Mass Spectrum SmartFormula Report

Analysis Info

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Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-155'160/f17	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

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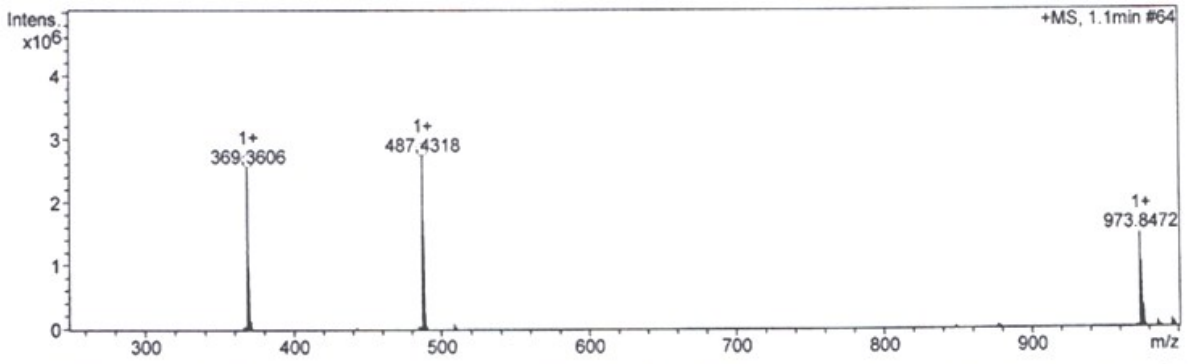
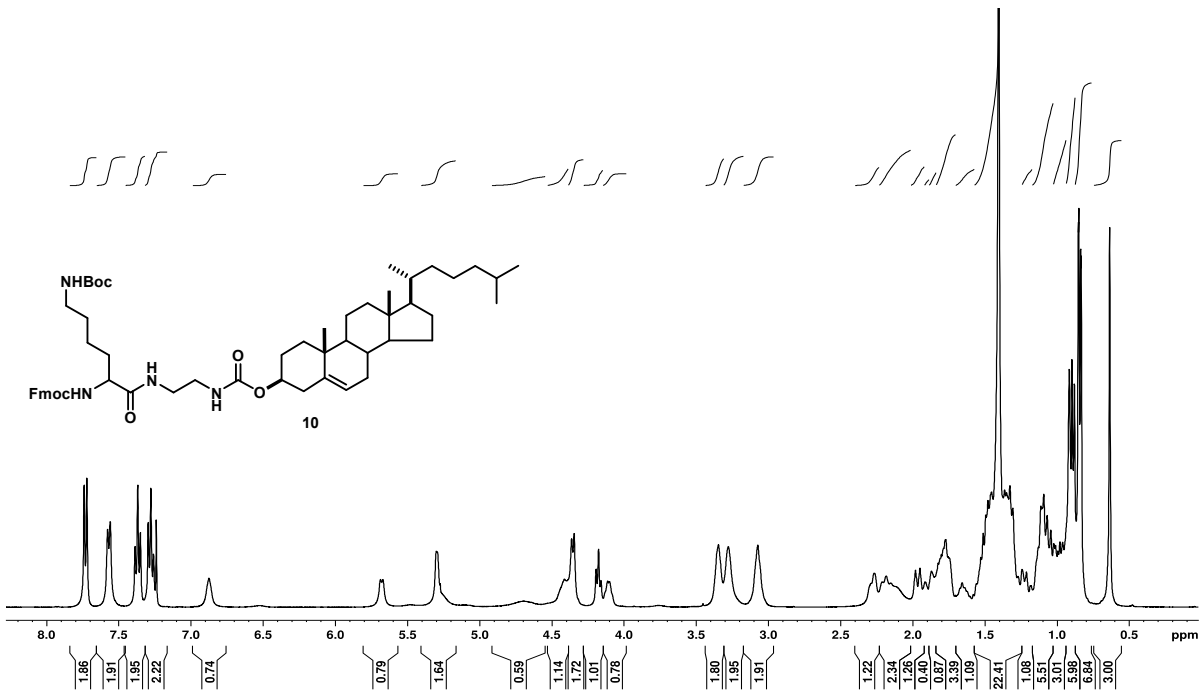
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



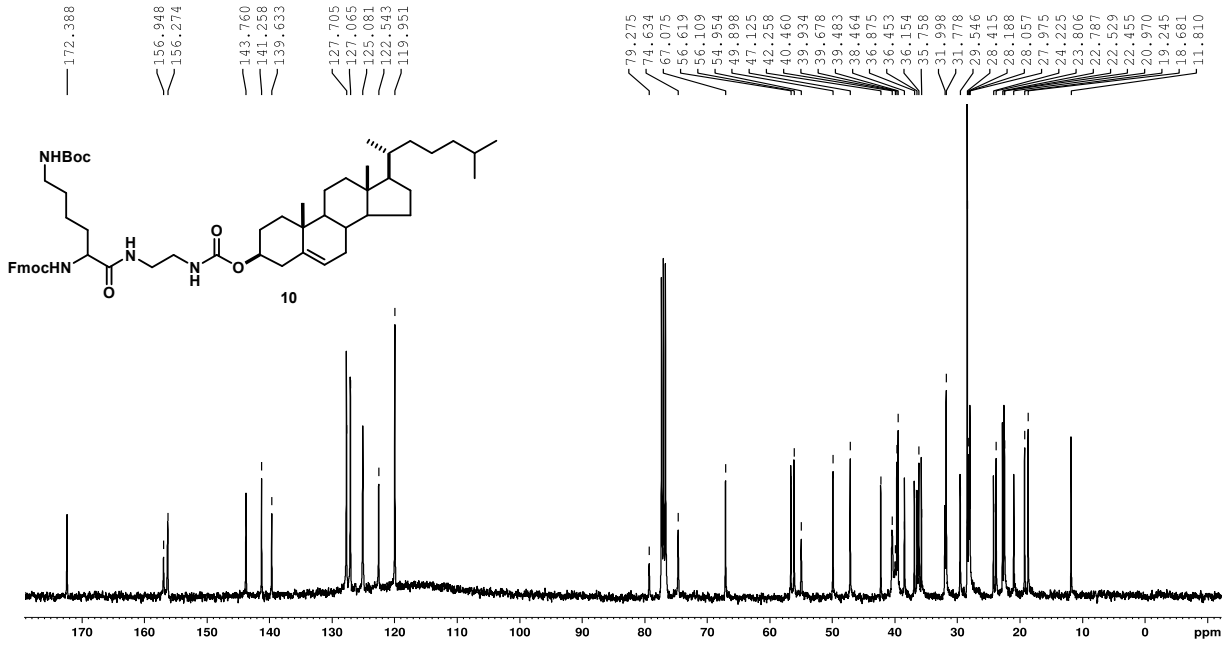
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
369.360621	1	C21H45N4O	369.358789	-5.0	-3.2	1.5	ok	even	80.4	114.9	1.5	47.5	2.1	842.7
	1	C21H45N4O	369.358789	-5.0	-3.2	1.5	ok	even	80.4	114.9	1.5	47.5	2.1	842.7
487.431774	1	C37H43	487.335928	196.6	-194.5	16.5	ok	even	70.2	86.8	94.9	26.9	2.6	873.7
	2	C36H55	487.429828	-4.0	-2.0	9.5	ok	even	75.1	94.1	1.6	29.1	2.6	531.4
	3	C35H67	487.523729	-188.7	190.5	2.5	ok	even	80.1	101.6	92.9	31.4	2.6	882.6
	4	C35H51O	487.393443	-78.6	-76.6	10.5	ok	even	80.7	102.0	37.4	31.6	2.6	778.8
	5	C34H51N2	487.404676	-55.6	-53.7	10.5	ok	even	82.9	105.9	26.2	32.7	2.4	744.6
	6	C34H63O	487.487343	-114.0	115.9	3.5	ok	even	85.6	109.6	56.6	33.9	2.6	833.4
	7	C34H47O2	487.357057	-153.3	-151.2	11.5	ok	even	86.2	110.0	73.8	34.1	2.5	863.6
	8	C33H63N2	487.498576	-137.0	138.8	3.5	ok	even	87.8	113.6	67.7	35.1	2.4	856.7
	9	C33H47N2O	487.368291	-130.2	-128.3	11.5	ok	even	88.4	114.0	62.6	35.3	2.4	848.4
	10	C33H59O2	487.450958	39.4	41.3	4.5	ok	even	91.1	117.7	20.2	36.5	2.5	733.7
	11	C32H59N2O	487.462191	-62.4	64.2	4.5	ok	even	93.3	121.8	31.3	37.7	2.4	781.4
	12	C32H43N2O2	487.331905	-204.9	-202.9	12.5	ok	even	93.9	122.1	99.0	37.9	2.3	902.4
	13	C32H55O3	487.414572	35.3	-33.3	5.5	ok	even	96.7	125.9	16.3	39.1	2.5	724.6
	14	C31H55N2O2	487.425805	12.2	-10.4	5.5	ok	even	98.9	130.0	5.2	40.3	2.4	647.5
	15	C30H63O4	487.472087	82.7	84.6	-0.5	ok	even	107.1	142.3	41.3	44.2	2.5	828.8

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:39:11 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-aminopropyl)carbamoyl]cholesterol (**9**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(Fmoc-lysin(Boc)amide))aminoethyl) carbamoyl]cholesterol (**10**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(Fmoc-lysin(Boc)amide))aminoethyl) carbamoyl]cholesterol (**10**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-124. (pos).d	Acquisition Date	9/10/2015 12:36:15 PM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-31	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
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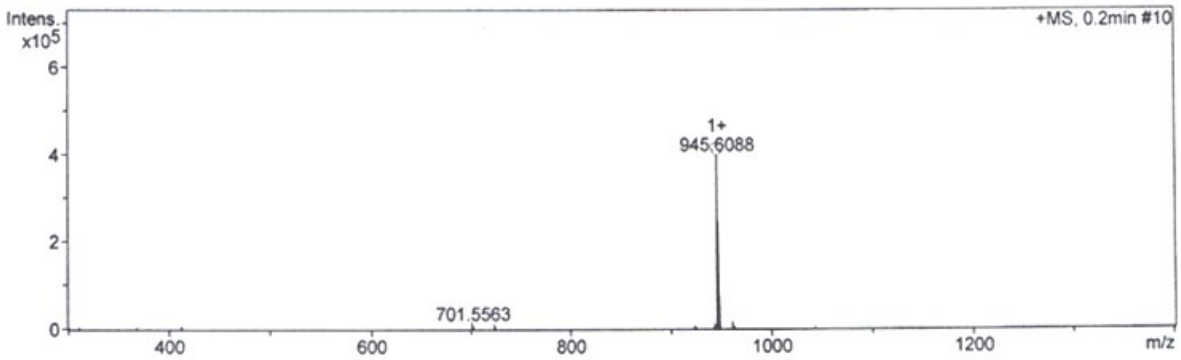
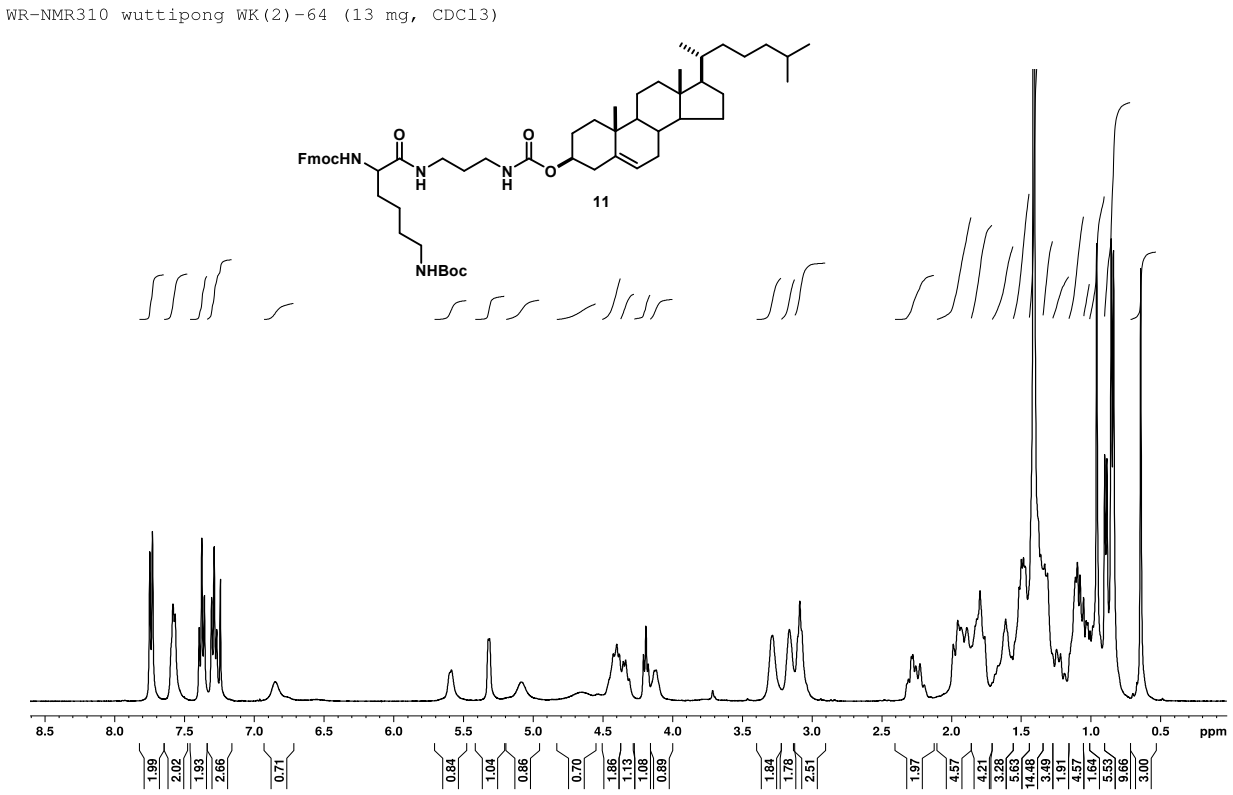
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



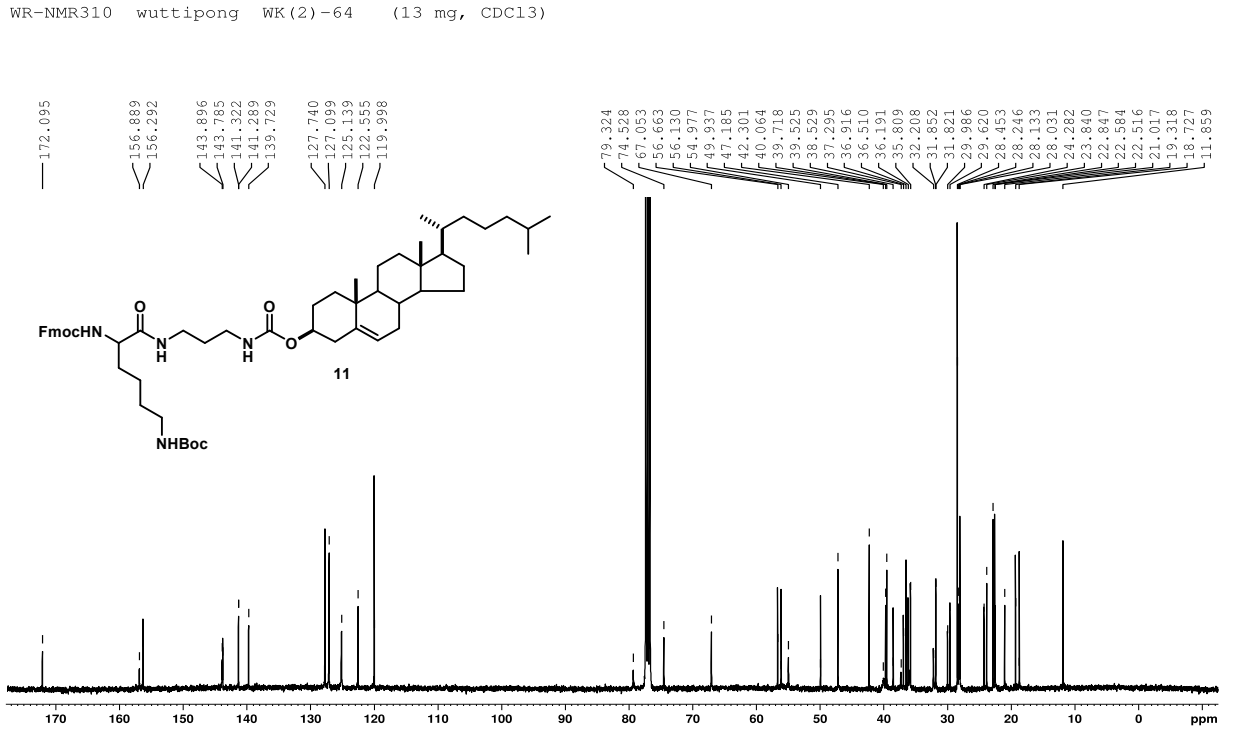
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I a	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
945.608849	1	C53H81N6O9	945.605955	3.1	-2.7	16.5	ok	even	5.0	5.4	2.6	1.5	0.8	934.1
	2	C57H85O11	945.608640	-0.2	0.2	15.5	ok	even	8.7	11.3	0.6	3.5	1.1	831.6
	3	C58H81N4O7	945.609977	-1.2	1.6	20.5	ok	even	18.6	20.4	1.6	5.7	1.0	951.2
	4	C70H77N2	945.608127	-0.8	-0.3	33.5	ok	even	81.1	80.1	0.7	22.5	1.2	987.4
	1	C53H81N6O9	945.605955	3.1	-2.7	16.5	ok	even	5.0	5.4	2.6	1.5	0.8	934.1
	2	C57H85O11	945.608640	-0.2	0.2	15.5	ok	even	8.7	11.3	0.6	3.5	1.1	831.6
	3	C58H81N4O7	945.609977	-1.2	1.6	20.5	ok	even	18.6	20.4	1.6	5.7	1.0	951.2
	4	C70H77N2	945.608127	-0.8	-0.3	33.5	ok	even	81.1	80.1	0.7	22.5	1.2	987.4
	1	C55H86NaO11	945.606234	-2.8	-2.3	12.5	ok	even	4.2	4.4	2.2	1.4	1.0	643.3
	2	C56H82N4NaO7	945.607572	1.4	-1.0	17.5	ok	even	7.1	7.7	1.0	2.1	0.9	560.7
	3	C61H82N2NaO5	945.611594	-2.9	3.4	21.5	ok	even	30.8	32.7	3.2	9.0	1.1	859.0
	4	C48H90NaO16	945.612108	-3.4	3.9	3.5	ok	even	39.5	45.0	3.7	12.0	0.9	898.1
	5	C68H78N2Na	945.605721	-3.3	-2.8	30.5	ok	even	68.9	68.8	2.8	19.1	1.2	917.6
	1	C70H77N2	945.608127	-0.8	-0.3	33.5	ok	even	81.1	80.1	0.7	22.5	1.2	987.4
	1	C48H90NaO16	945.612108	-3.4	3.9	3.5	ok	even	39.5	45.0	3.7	12.0	0.9	898.1
	2	C68H78N2Na	945.605721	-3.3	-2.8	30.5	ok	even	68.9	68.8	2.8	19.1	1.2	917.6

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:27:50 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N'*-(Fmoc-lysin(Boc)amide))aminoethyl)carbamoyl] cholesterol (**10**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(Fmoc-lysin(Boc)amide))aminopropyl) carbamoyl]cholesterol (**11**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(Fmoc-lysin(Boc)amide))aminopropyl) carbamoyl]cholesterol (**11**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-147 (pos).d	Acquisition Date	1/21/2016 11:26:10 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-170/55-59	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
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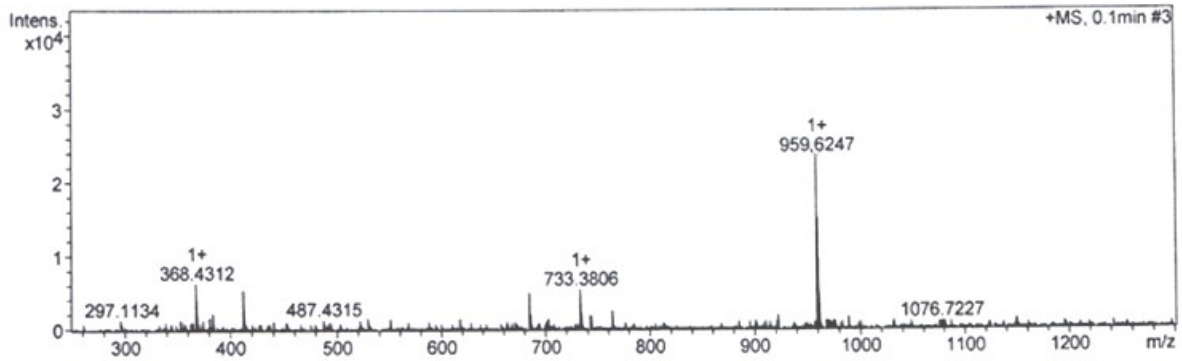
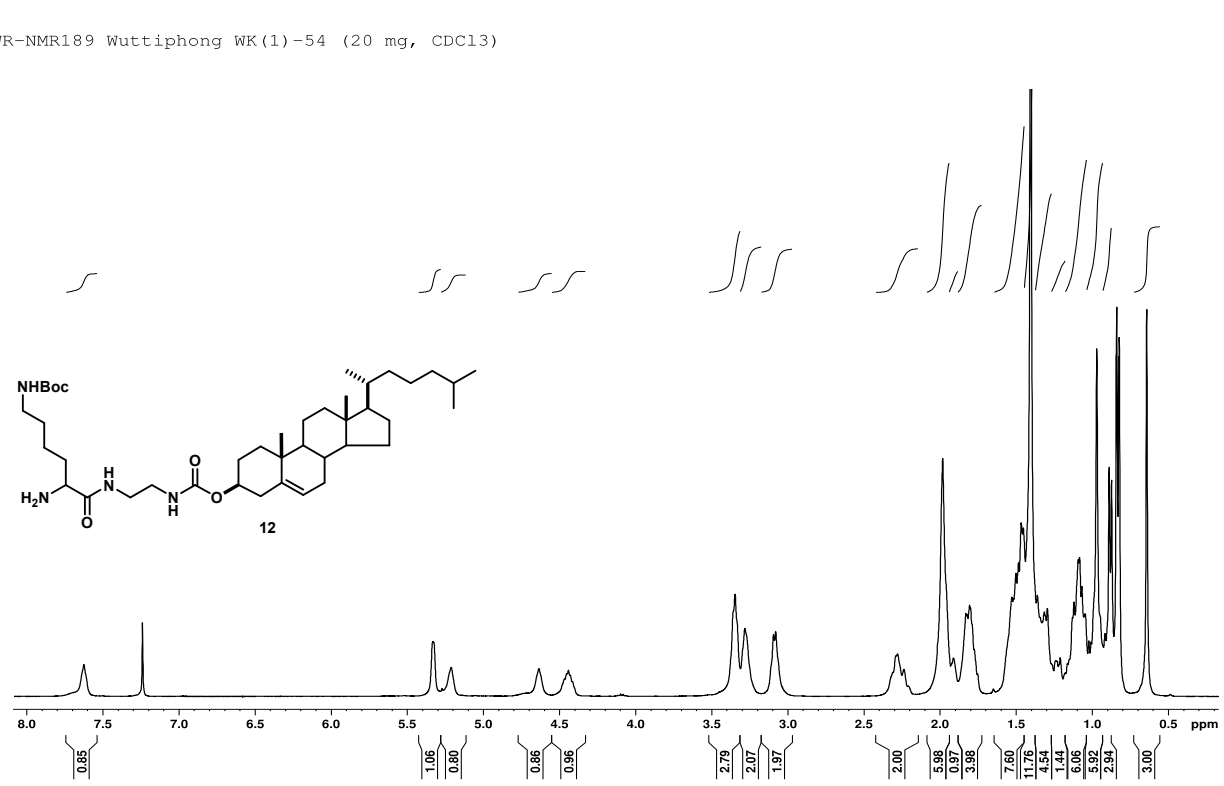
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

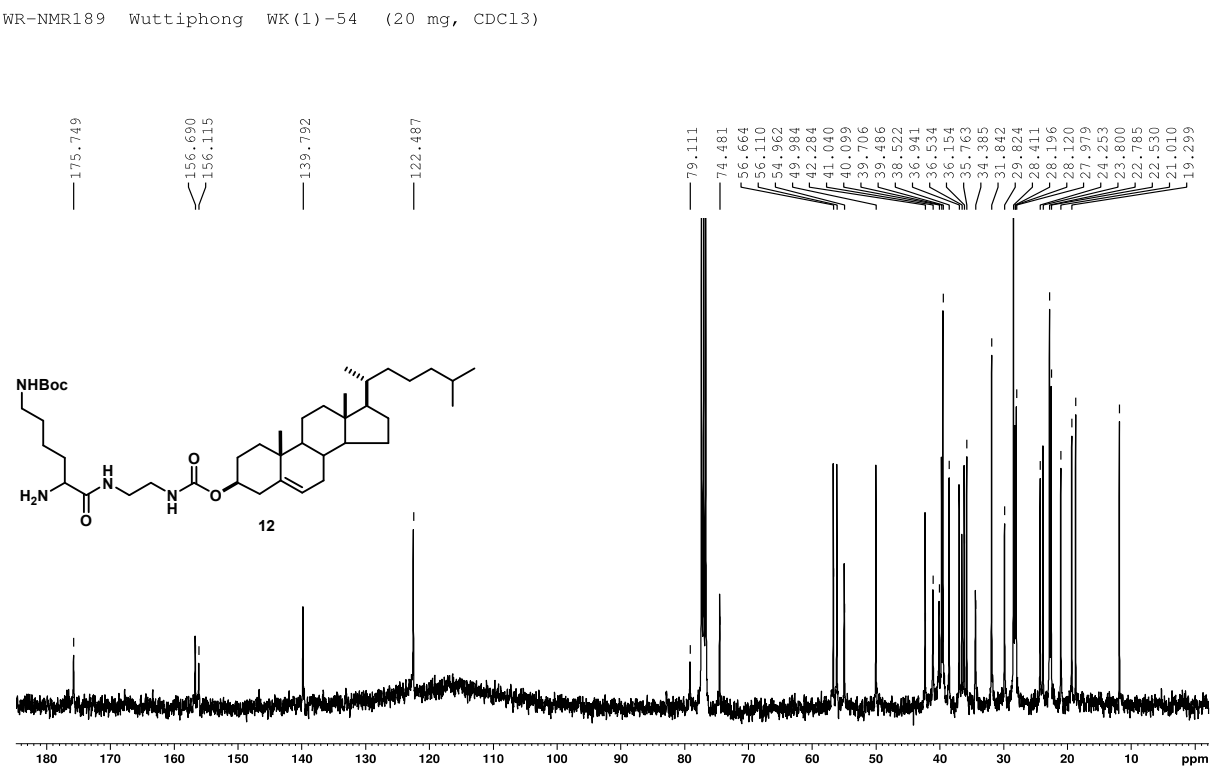


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
959.624708	1	C58H87O11	959.624290	0.4	-0.9	15.5	ok	even	4.1	5.5	1.1	3.0	1.4	709.1
	2	C57H84N4NaO7	959.623222	-1.5	-2.1	17.5	ok	even	6.6	9.9	2.2	4.2	1.5	821.2
	3	C59H83N4O7	959.625627	1.0	0.4	20.5	ok	even	11.4	11.1	0.9	4.1	1.5	722.0
	4	C56H88NaO11	959.621884	-2.9	-3.4	12.5	ok	even	13.0	15.8	3.4	5.2	1.4	887.7
	5	C62H84N2NaO5	959.627245	-2.6	2.2	21.5	ok	even	23.0	21.9	2.2	6.6	1.5	869.6
	6	C49H92NaO16	959.627758	-3.2	2.7	3.5	ok	even	48.2	55.8	2.7	15.3	1.3	954.6
	7	C69H80N2Na	959.621371	-3.5	-3.9	30.5	ok	even	60.7	56.4	3.9	15.8	1.6	970.9
	8	C71H79N2	959.623777	1.0	-1.4	33.5	ok	even	72.9	67.3	1.6	18.9	1.6	950.0
	1	C58H87O11	959.624290	0.4	-0.9	15.5	ok	even	4.1	5.5	1.1	3.0	1.4	709.1
	2	C57H84N4NaO7	959.623222	-1.5	-2.1	17.5	ok	even	6.6	9.9	2.2	4.2	1.5	821.2
	3	C59H83N4O7	959.625627	1.0	0.4	20.5	ok	even	11.4	11.1	0.9	4.1	1.5	722.0
	4	C56H88NaO11	959.621884	-2.9	-3.4	12.5	ok	even	13.0	15.8	3.4	5.2	1.4	887.7
	5	C62H84N2NaO5	959.627245	-2.6	2.2	21.5	ok	even	23.0	21.9	2.2	6.6	1.5	869.6
	6	C49H92NaO16	959.627758	-3.2	2.7	3.5	ok	even	48.2	55.8	2.7	15.3	1.3	954.6
	7	C69H80N2Na	959.621371	-3.5	-3.9	30.5	ok	even	60.7	56.4	3.9	15.8	1.6	970.9
	8	C71H79N2	959.623777	1.0	-1.4	33.5	ok	even	72.9	67.3	1.6	18.9	1.6	950.0
	1	C57H84N4NaO7	959.623222	-1.5	-2.1	17.5	ok	even	6.6	9.9	2.2	4.2	1.5	770.7
	2	C60H85N2Na2O5	959.624839	0.1	-0.4	18.5	ok	even	12.6	12.5	0.9	4.6	1.5	677.9
	3	C56H88NaO11	959.621884	-2.9	-3.4	12.5	ok	even	13.0	15.8	3.4	5.2	1.4	844.6
	4	C55H85N4Na2O7	959.620816	4.1	-4.6	14.5	ok	even	15.0	20.0	4.5	6.6	1.4	896.5
	5	C62H84N2NaO5	959.627245	-2.6	2.2	21.5	ok	even	23.0	21.9	2.2	6.6	1.5	823.4
	6	C49H92NaO16	959.627758	-3.2	2.7	3.5	ok	even	48.2	55.8	2.7	15.3	1.3	925.8
	7	C69H80N2Na	959.621371	-3.5	-3.9	30.5	ok	even	60.7	56.4	3.9	15.8	1.6	948.6

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N*′-(Fmoc-lysin(Boc)amide))aminopropyl)carbamoyl] cholesterol (**11**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(lysin(Boc)amide))aminoethyl) carbamoyl]cholesterol (**12**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(lysin(Boc)amide))aminoethyl) carbamoyl]cholesterol (**12**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-123. (pos).d	Acquisition Date	9/10/2015 12:32:57 PM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-54	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
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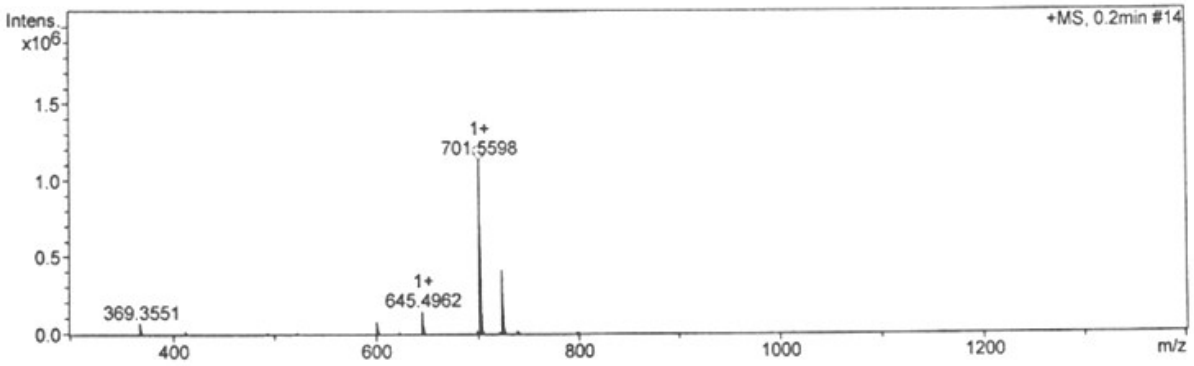
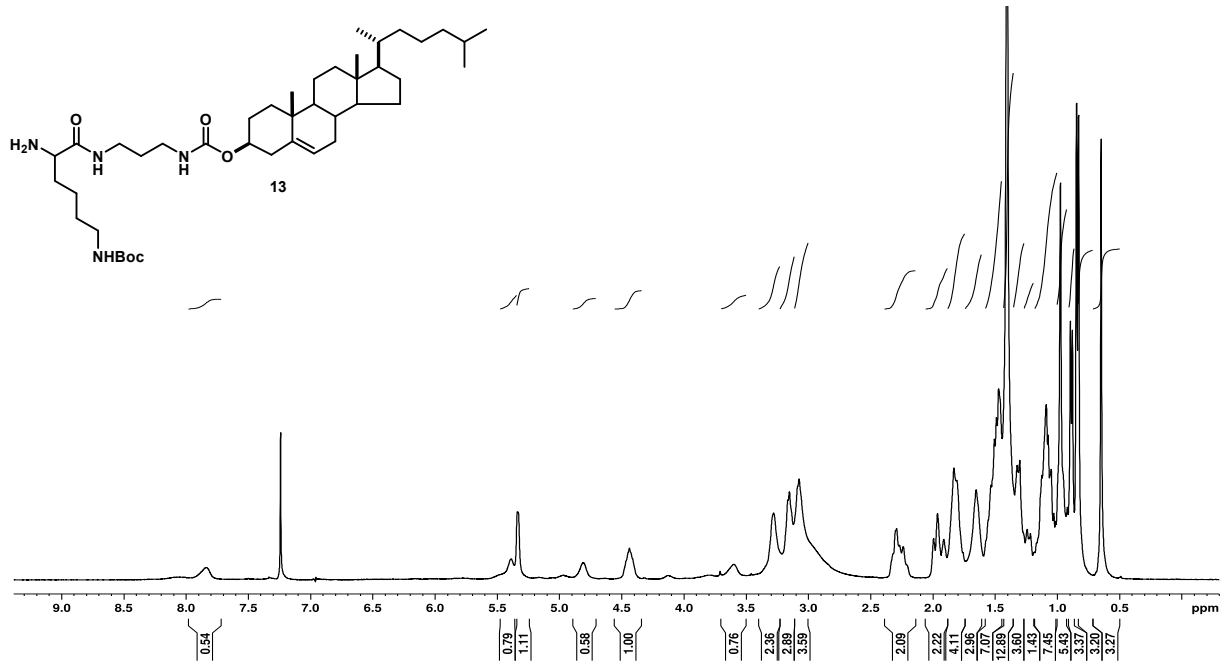
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

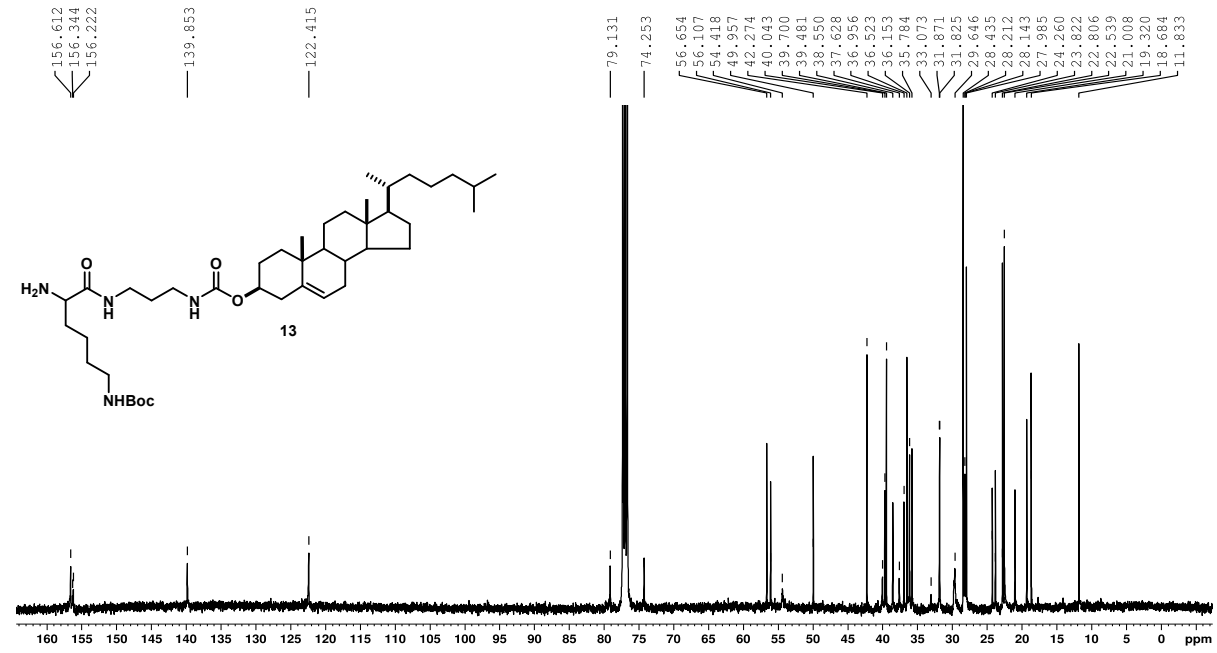


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
701.559839	1	C46H73N2O3	701.561571	-2.5	3.0	11.5	ok	even	12.2	16.5	2.2	6.0	1.1	842.7
	2	C41H73N4O5	701.557548	3.3	-2.8	7.5	ok	even	14.5	16.9	2.0	5.4	0.9	825.0
	3	C40H77O9	701.556210	-5.2	-4.6	2.5	ok	even	26.3	31.1	3.3	10.0	1.0	925.8
	1	C46H73N2O3	701.561571	-2.5	3.0	11.5	ok	even	12.2	16.5	2.2	6.0	1.1	842.7
	2	C41H73N4O5	701.557548	3.3	-2.8	7.5	ok	even	14.5	16.9	2.0	5.4	0.9	825.0
	3	C40H77O9	701.556210	-5.2	-4.6	2.5	ok	even	26.3	31.1	3.3	10.0	1.0	925.8
	1	C44H74N2NaO3	701.559165	1.0	-0.5	8.5	ok	even	3.9	5.0	0.6	2.2	1.1	842.7
	2	C49H74NaO	701.563188	-4.8	5.3	12.5	ok	even	24.5	30.6	3.8	10.5	1.2	999.1

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N*′-(lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (**12**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(lysin(Boc)amide))aminopropyl) carbamoyl]cholesterol (**13**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(lysin(Boc)amide))aminopropyl) carbamoyl]cholesterol (**13**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-148 (pos).d	Acquisition Date	1/21/2016 11:31:21 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-181/12-14	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
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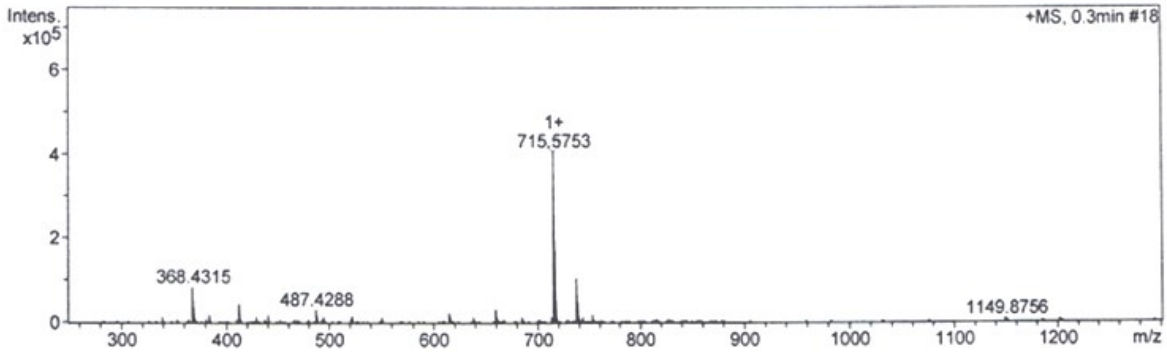
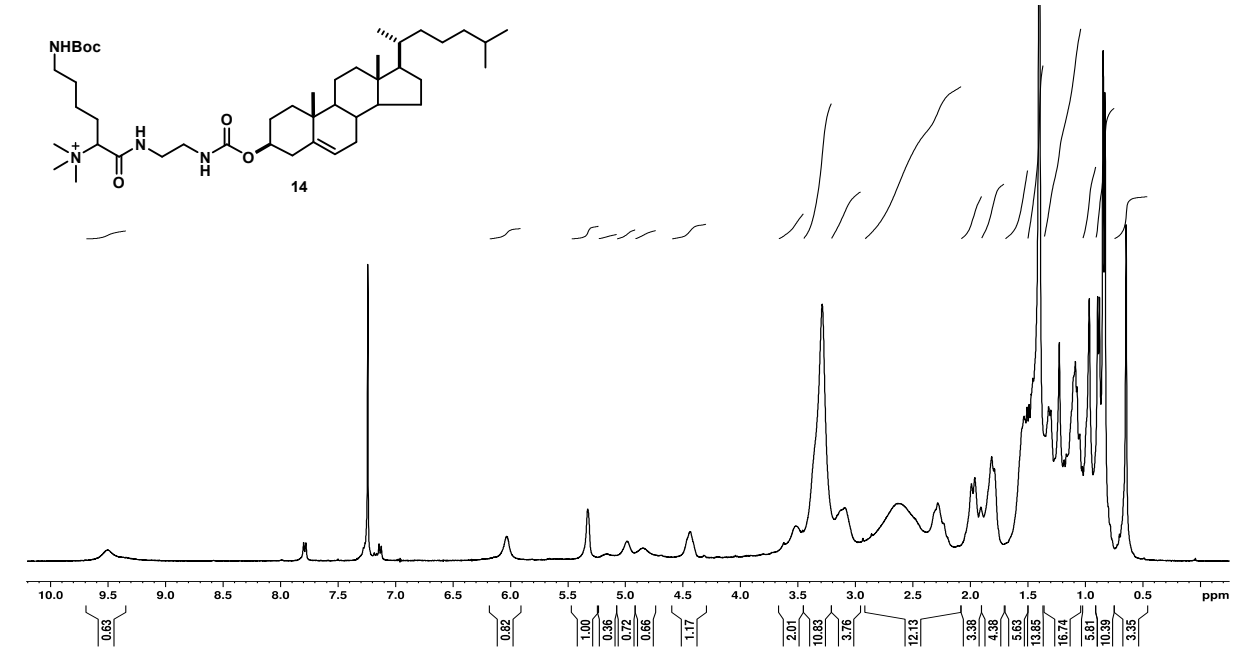
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



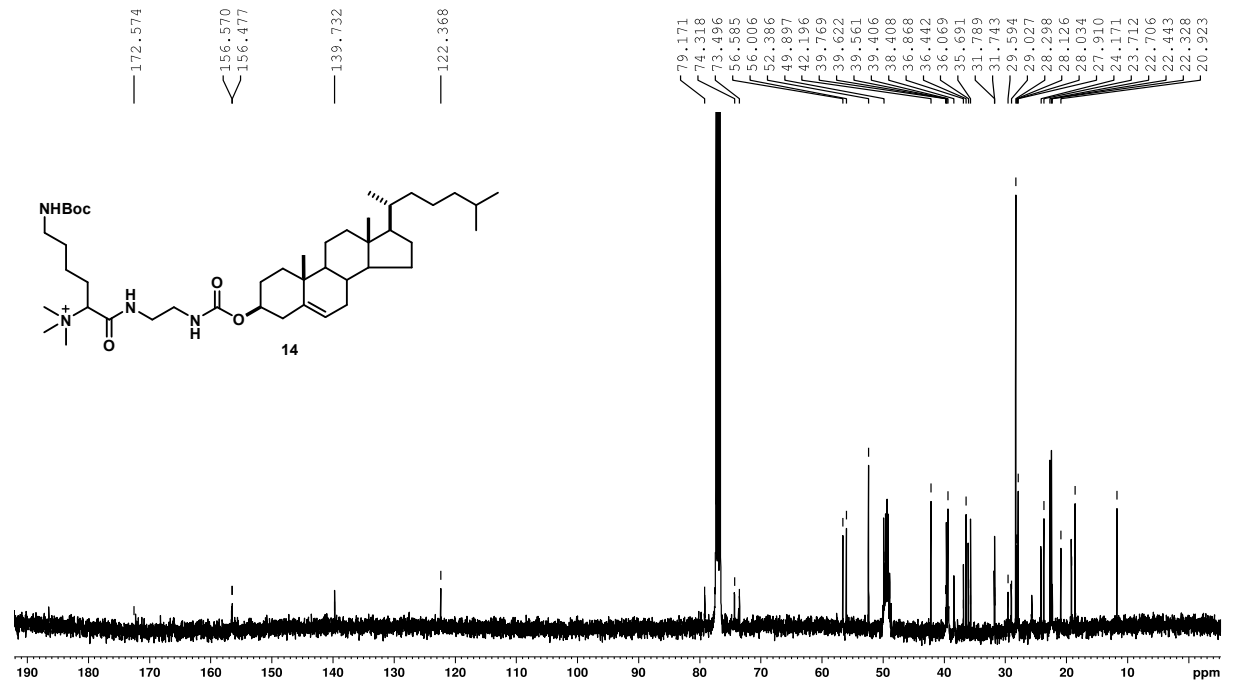
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I a	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
715.575290	1	C42H75N4O5	715.573198	2.9	-2.3	7.5	ok	even	1.8	2.3	1.8	1.2	1.0	591.2
	2	C45H76N2NaO3	715.574815	0.7	0.1	8.5	ok	even	11.8	14.1	0.7	4.7	1.1	683.6
	3	C41H79O9	715.571861	-4.8	-3.7	2.5	ok	even	12.1	17.2	3.9	4.8	1.3	842.7
	4	C47H75N2O3	715.577221	-2.7	3.7	11.5	ok	even	21.6	27.9	3.9	7.8	1.3	892.0
	5	C50H76NaO	715.578838	-5.0	6.1	12.5	ok	even	33.1	42.4	5.2	11.4	1.5	943.4
	1	C42H75N4O5	715.573198	2.9	-2.3	7.5	ok	even	1.8	2.3	1.8	1.2	1.0	591.2
	2	C45H76N2NaO3	715.574815	0.7	0.1	8.5	ok	even	11.8	14.1	0.7	4.7	1.1	683.6
	3	C41H79O9	715.571861	-4.8	-3.7	2.5	ok	even	12.1	17.2	3.9	4.8	1.3	842.7
	4	C47H75N2O3	715.577221	-2.7	3.7	11.5	ok	even	21.6	27.9	3.9	7.8	1.3	892.0
	5	C50H76NaO	715.578838	-5.0	6.1	12.5	ok	even	33.1	42.4	5.2	11.4	1.5	943.4
	1	C43H77N2Na2O3	715.572409	4.0	-3.3	5.5	ok	even	2.2	3.4	2.5	1.9	1.1	794.1
	2	C45H76N2NaO3	715.574815	0.7	0.1	8.5	ok	even	11.8	14.1	0.7	4.7	1.1	748.3
	3	C48H77Na2O	715.576432	-1.6	2.4	9.5	ok	even	24.7	29.2	1.9	9.5	1.2	913.8
	4	C50H76NaO	715.578838	-5.0	6.1	12.5	ok	even	33.1	42.4	5.2	11.4	1.5	977.5

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:44:22 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N*′-(lysin(Boc)amide))aminopropyl)carbamoyl]cholesterol (**13**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (**14**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysin(Boc)amide))aminoethyl)carbamoyl]cholesterol (**14**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-142 (pos).d	Acquisition Date	1/21/2016 11:04:23 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-133/7-8	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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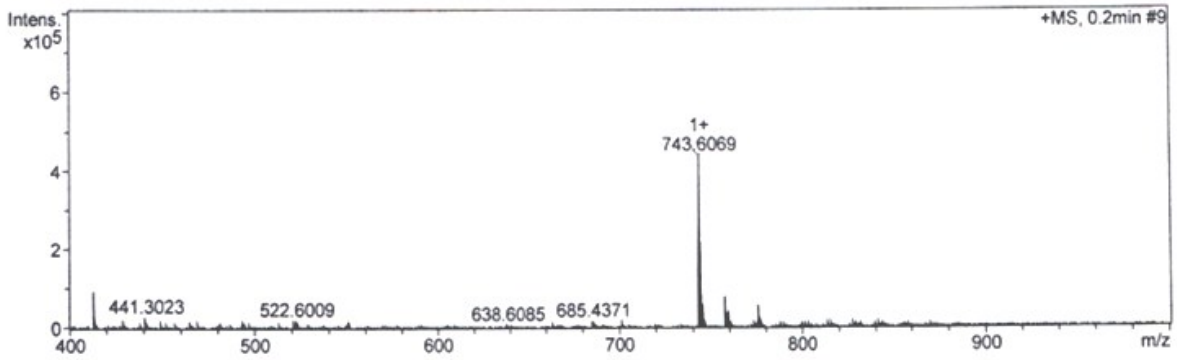
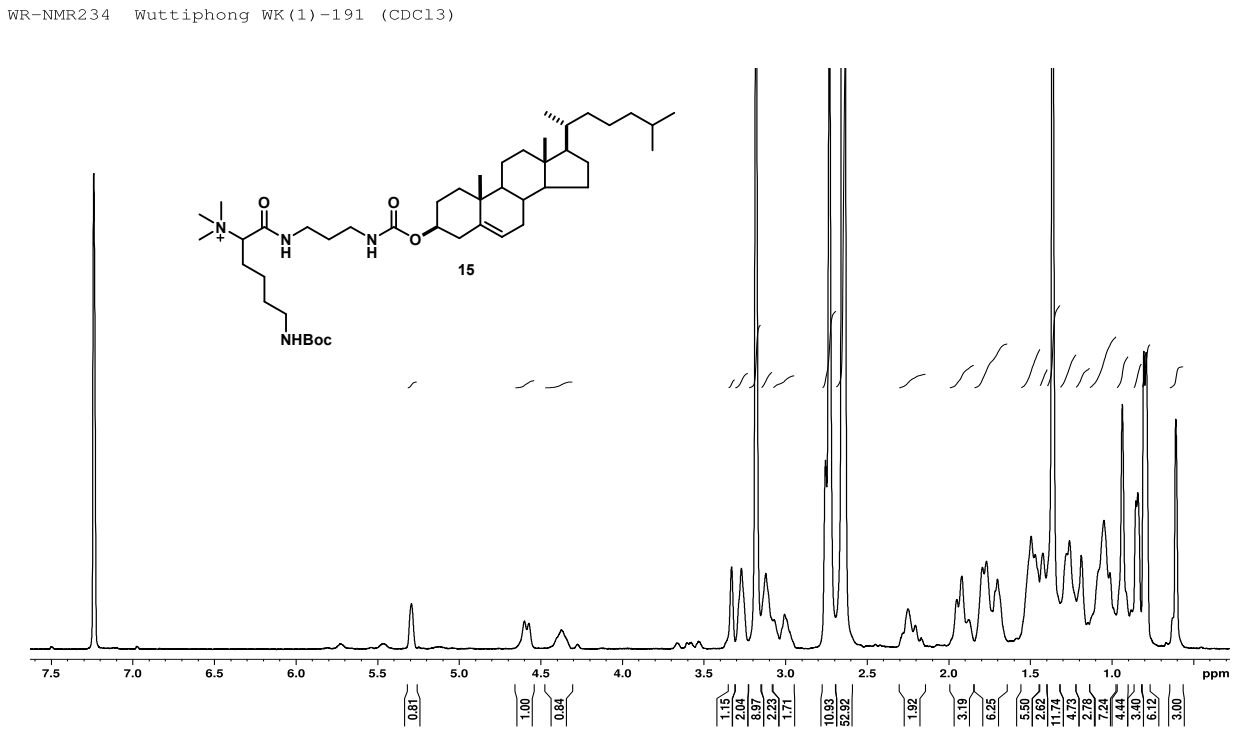
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

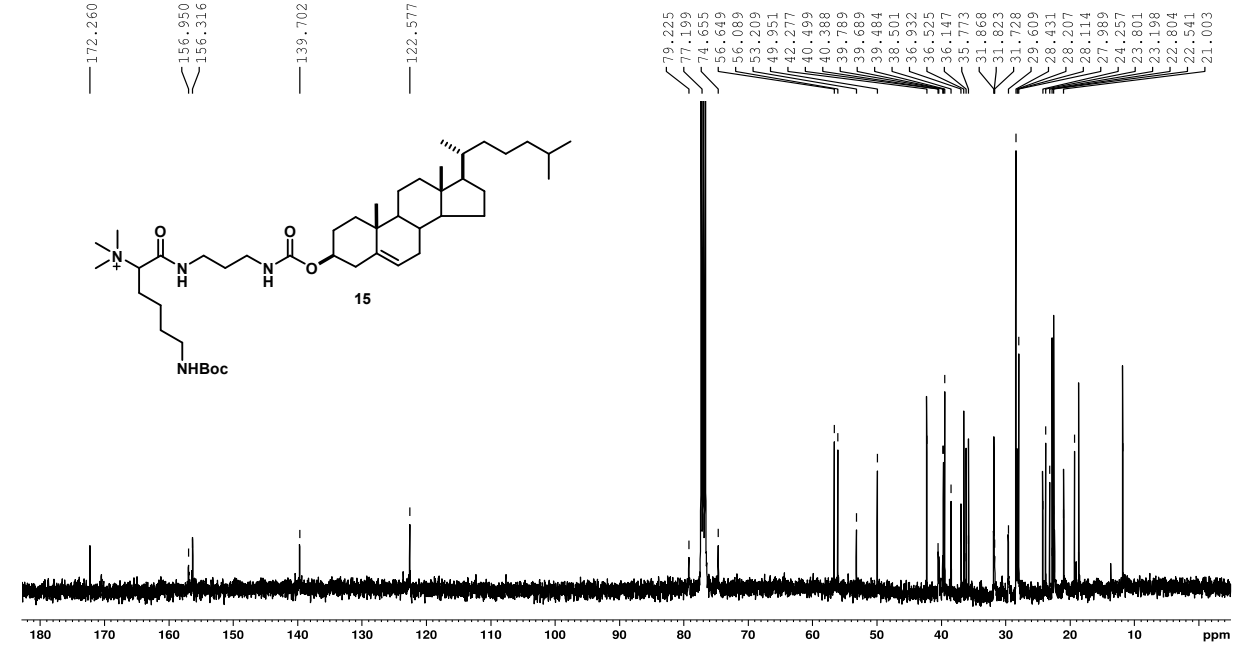


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
743.606895	1	C44H79N4O5	743.604498	3.2	-2.2	7.5	ok	even	7.1	10.3	4.2	4.4	1.0	833.2
	2	C43H83O9	743.603161	-5.0	-3.9	2.5	ok	even	7.4	12.8	4.9	4.4	1.1	848.0
	3	C49H79N2O3	743.608521	-2.2	3.3	11.5	ok	even	27.1	32.7	4.6	9.1	1.2	915.1
	1	C44H79N4O5	743.604498	3.2	-2.2	7.5	ok	even	7.1	10.3	4.2	4.4	1.0	833.2
	2	C43H83O9	743.603161	-5.0	-3.9	2.5	ok	even	7.4	12.8	4.9	4.4	1.1	848.0
	3	C49H79N2O3	743.608521	-2.2	3.3	11.5	ok	even	27.1	32.7	4.6	9.1	1.2	915.1
	1	C47H80N2NaO3	743.606115	1.0	0.0	8.5	ok	even	16.9	20.2	4.0	6.3	1.2	842.7
	2	C52H80NaO	743.610138	-4.4	5.5	12.5	ok	even	38.7	46.6	5.7	12.6	1.3	935.7

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysin(Boc)amide)) aminoethyl)carbamoyl]cholesterol (**14**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysin(Boc)amide))aminopropyl)carbamoyl]cholesterol (**15**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysin(Boc)amide))aminopropyl)carbamoyl]cholesterol (**15**)

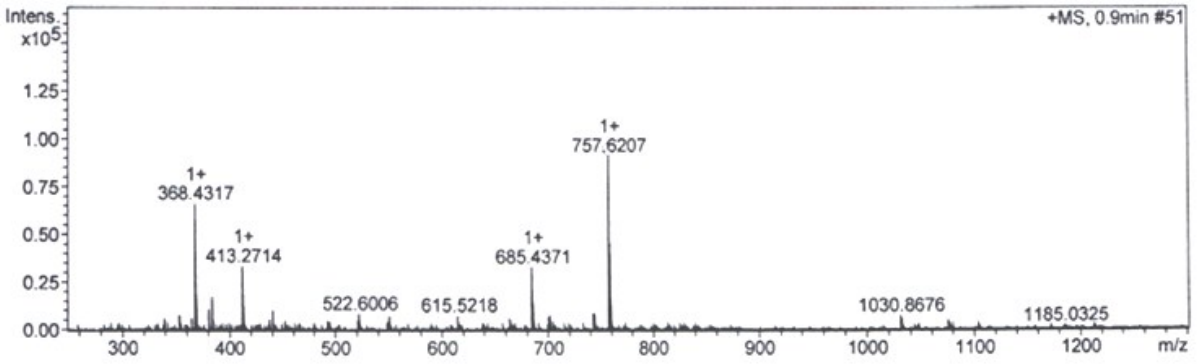
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-150 (pos).d	Acquisition Date	1/21/2016 11:41:14 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK(1)-191/14(2)/F16-F25	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

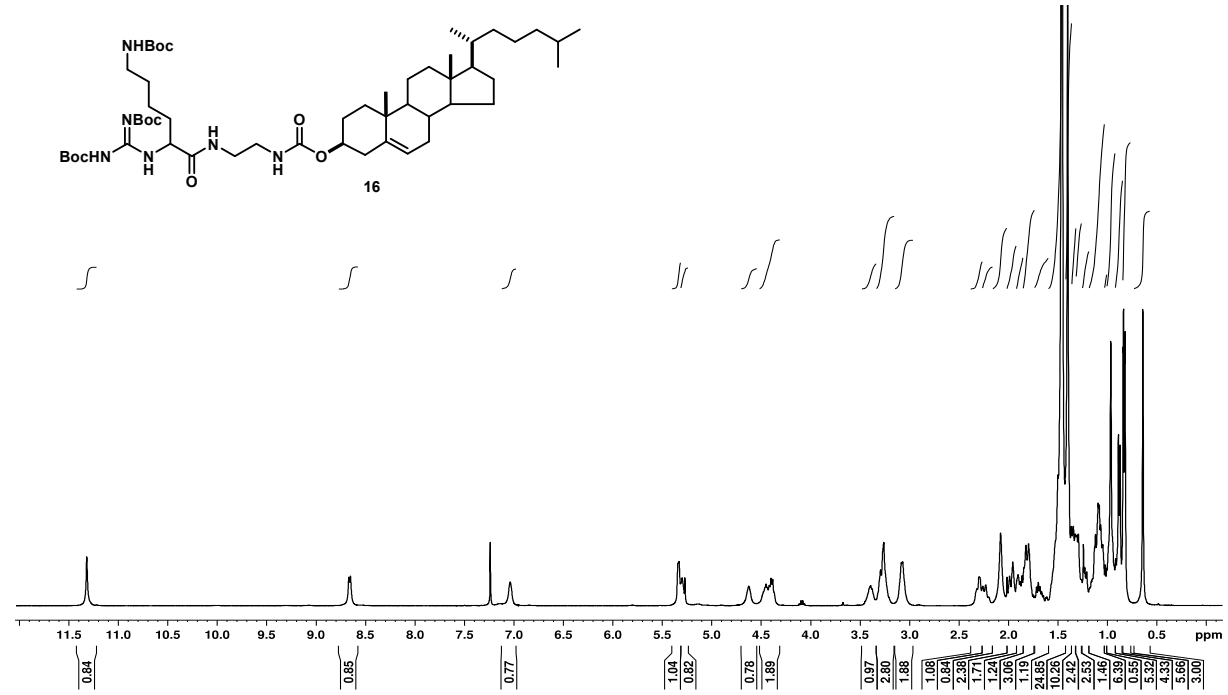
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



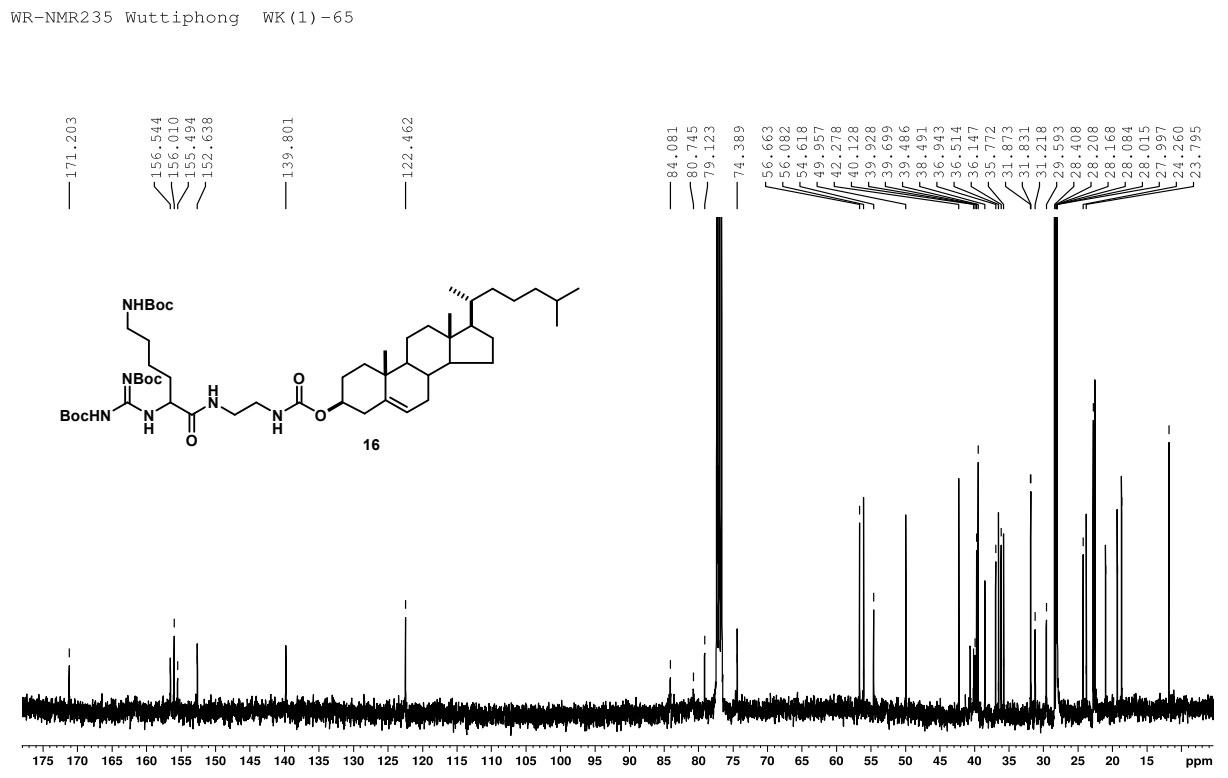
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I a	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
757.620672	1	C45H81N4O5	757.620148	-0.7	-0.6	7.5	ok	even	7.2	10.4	0.8	4.3	1.4	713.3
	2	C44H85O9	757.618811	2.5	-2.3	2.5	ok	even	8.7	13.9	1.8	4.6	1.4	818.7
	3	C43H82N4NaO5	757.617742	3.9	-3.8	4.5	ok	even	12.3	18.7	2.9	8.0	1.2	915.3
	4	C48H82N2NaO3	757.621765	-1.4	1.6	8.5	ok	even	16.0	19.0	1.4	5.8	1.5	813.0
	5	C50H81N2O3	757.624171	-4.6	4.8	11.5	ok	even	25.9	31.0	3.7	8.4	1.5	935.8
	1	C45H81N4O5	757.620148	-0.7	-0.6	7.5	ok	even	7.2	10.4	0.8	4.3	1.4	713.3
	2	C44H85O9	757.618811	2.5	-2.3	2.5	ok	even	8.7	13.9	1.8	4.6	1.4	818.7
	3	C43H82N4NaO5	757.617742	3.9	-3.8	4.5	ok	even	12.3	18.7	2.9	8.0	1.2	915.3
	4	C48H82N2NaO3	757.621765	-1.4	1.6	8.5	ok	even	16.0	19.0	1.4	5.8	1.5	813.0
	5	C50H81N2O3	757.624171	-4.6	4.8	11.5	ok	even	25.9	31.0	3.7	8.4	1.5	935.8
	1	C46H83N2Na2O3	757.619360	-1.7	-1.6	5.5	ok	even	9.9	12.9	1.4	6.4	1.4	842.2
	2	C43H82N4NaO5	757.617742	3.9	-3.8	4.5	ok	even	12.3	18.7	2.9	8.0	1.2	931.2
	3	C48H82N2NaO3	757.621765	-1.4	1.6	8.5	ok	even	16.0	19.0	1.4	5.8	1.5	831.8
	4	C51H83Na2O	757.623382	-3.6	3.8	9.5	ok	even	27.1	32.0	3.0	8.7	1.7	937.2

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:46:23 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysin(Boc)amide)) aminopropyl)carbamoyl]cholesterol (**15**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide))))aminoethyl)carbamoyl]cholesterol (**16**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide))))aminoethyl)carbamoyl]cholesterol (**16**)

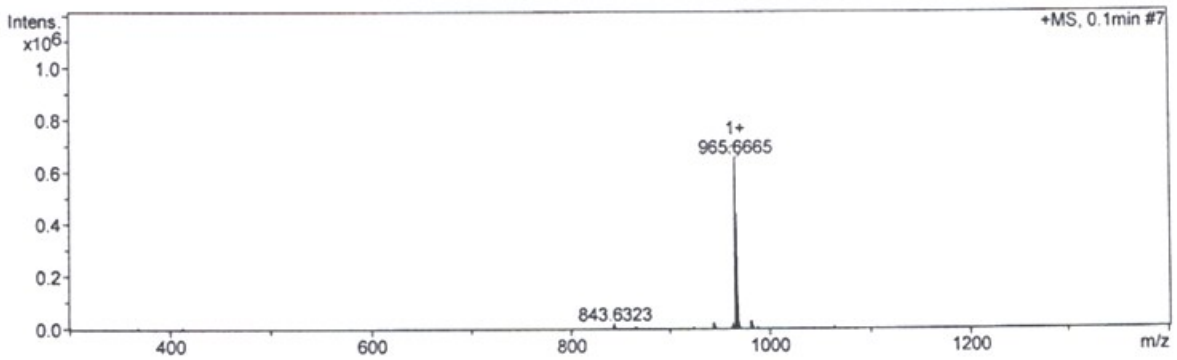
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-120. (pos).d	Acquisition Date	9/10/2015 12:17:33 PM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-65	Instrument	micrOTOF 8213750.10411
Comment			

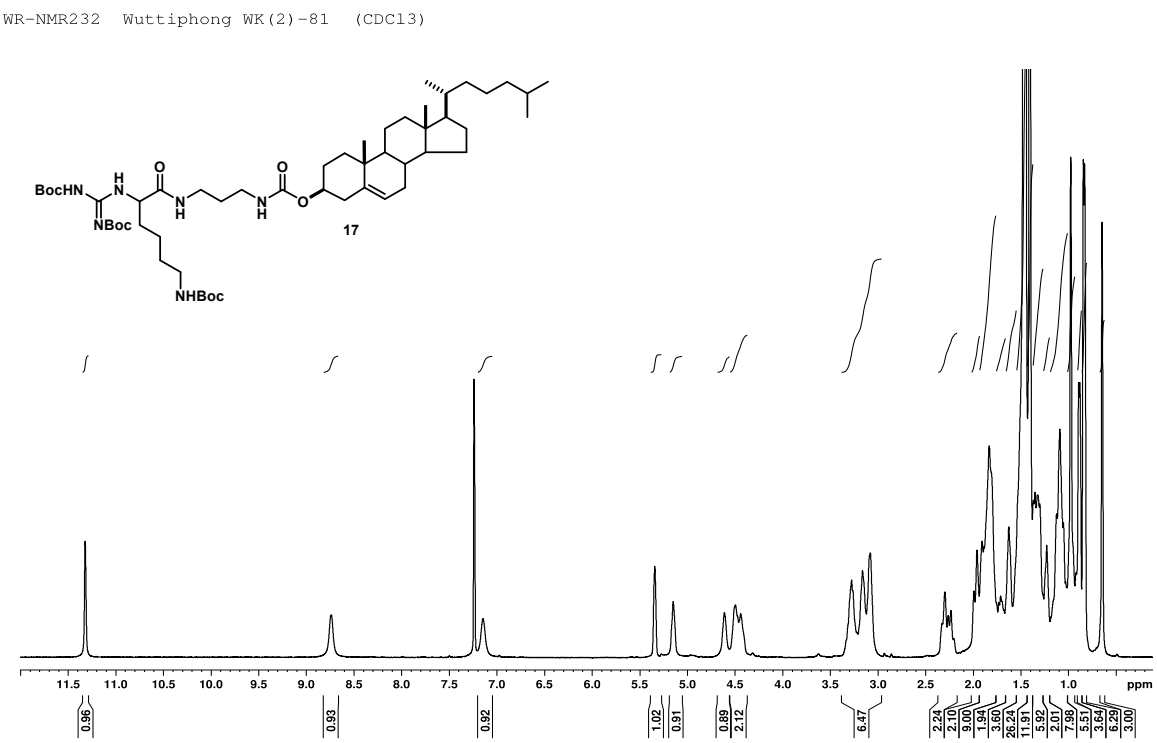
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

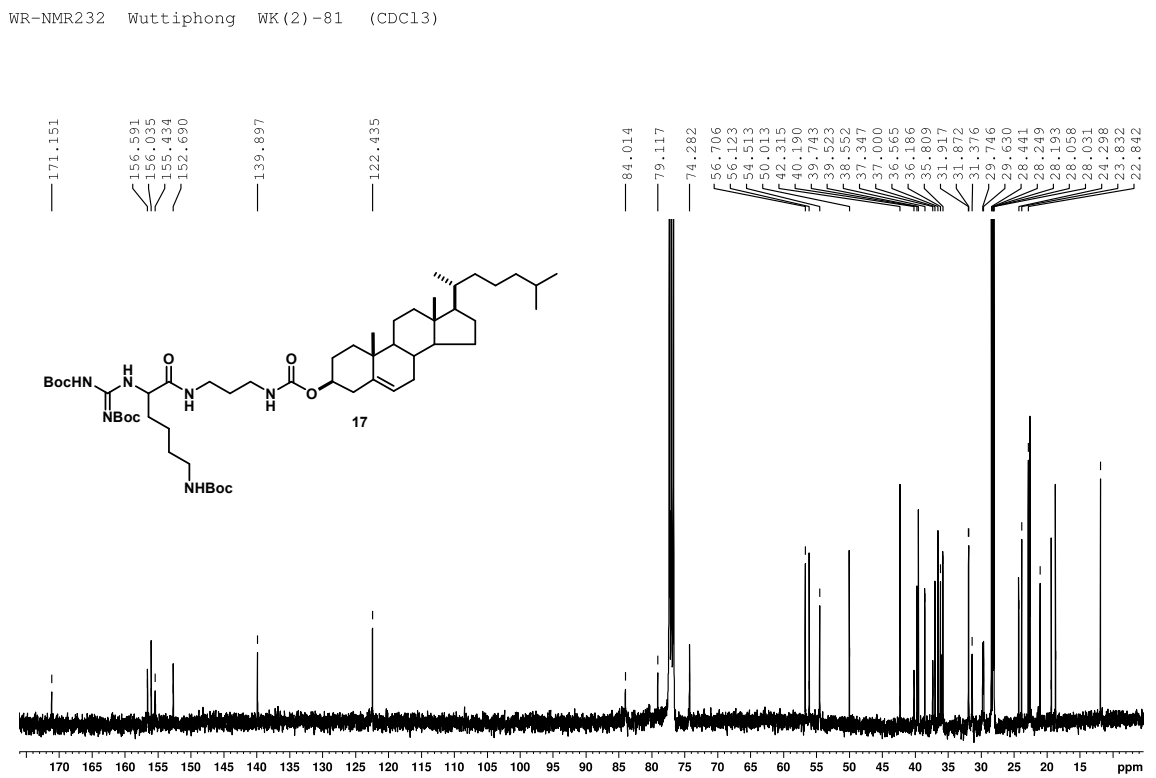


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
965.666542	1	C52H90N6NaO9	965.666149	-0.4	-0.0	10.5	ok	even	35.9	36.7	0.5	9.7	0.6	744.9

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide)))) aminoethyl)carbamoyl]cholesterol (**16**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide))))aminopropyl)carbamoyl]cholesterol (**17**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*-(*N‴*,*N‴*-diBoc(guanidinyl-lysin(Boc)amide))))aminopropyl)carbamoyl]cholesterol (**17**)

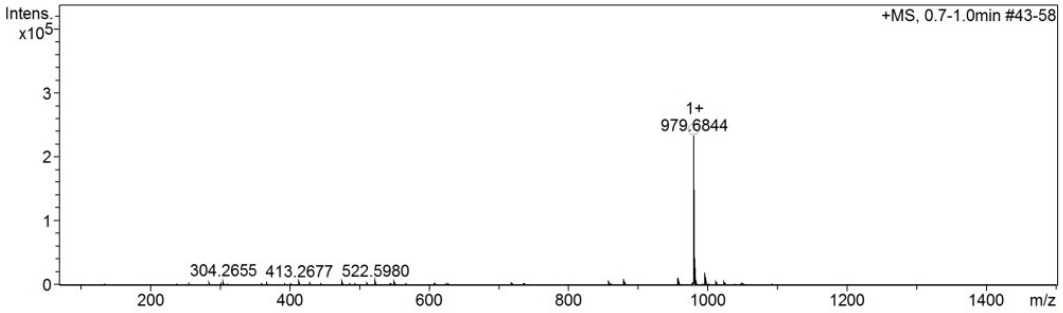
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS 579 (pos).d	Acquisition Date	9/13/2018 10:32:47 AM
Method	tune_low.m	Operator	RU
Sample Name	PK(1)-4	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

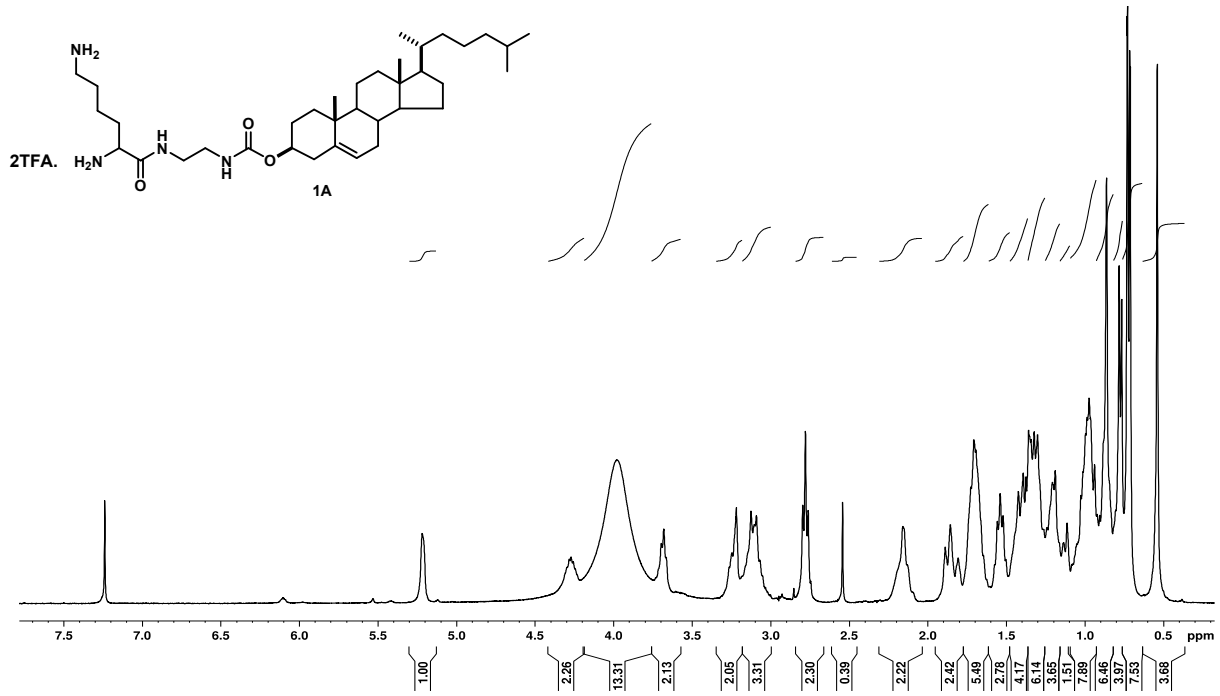
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



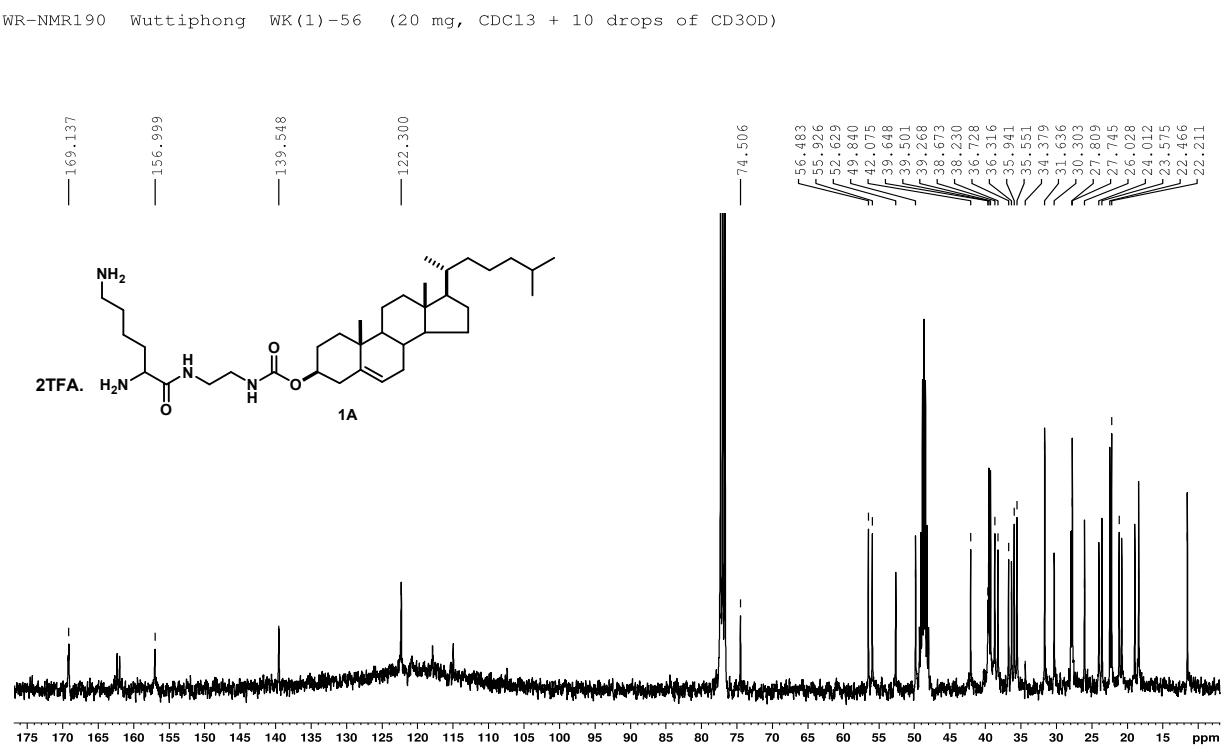
Meas. m/z	#	Ion Formula	m/z	|err| [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma
979.684399	1	C53H92N6NaO9	979.681799	2.6	2.7	-2.3	10.5	ok	even	13.8
	2	C54H88N10NaO5	979.683137	1.3	-1.3	-1.0	15.5	ok	even	23.4
	3	C46H96N6NaO14	979.687672	3.3	3.3	3.6	1.5	ok	even	24.4

Bruker Compass DataAnalysis 4.1 printed: 11/20/2018 12:38:34 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N*′-(*N*″-(*N*‴,*N*‴-diBoc(guanidinyl-lysin(Boc)amide)))) aminopropyl)carbamoyl]cholesterol (**17**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N'*-lysinamide)aminoethyl)carbamoyl] cholesterol (**1A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N'*-lysinamide)aminoethyl)carbamoyl] cholesterol (**1A**)

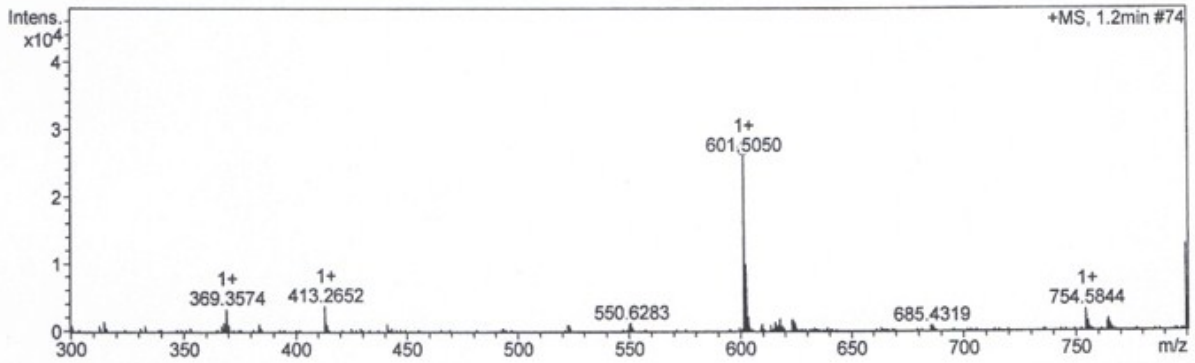
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-122. (pos).d	Acquisition Date	9/10/2015 12:23:46 PM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-56	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

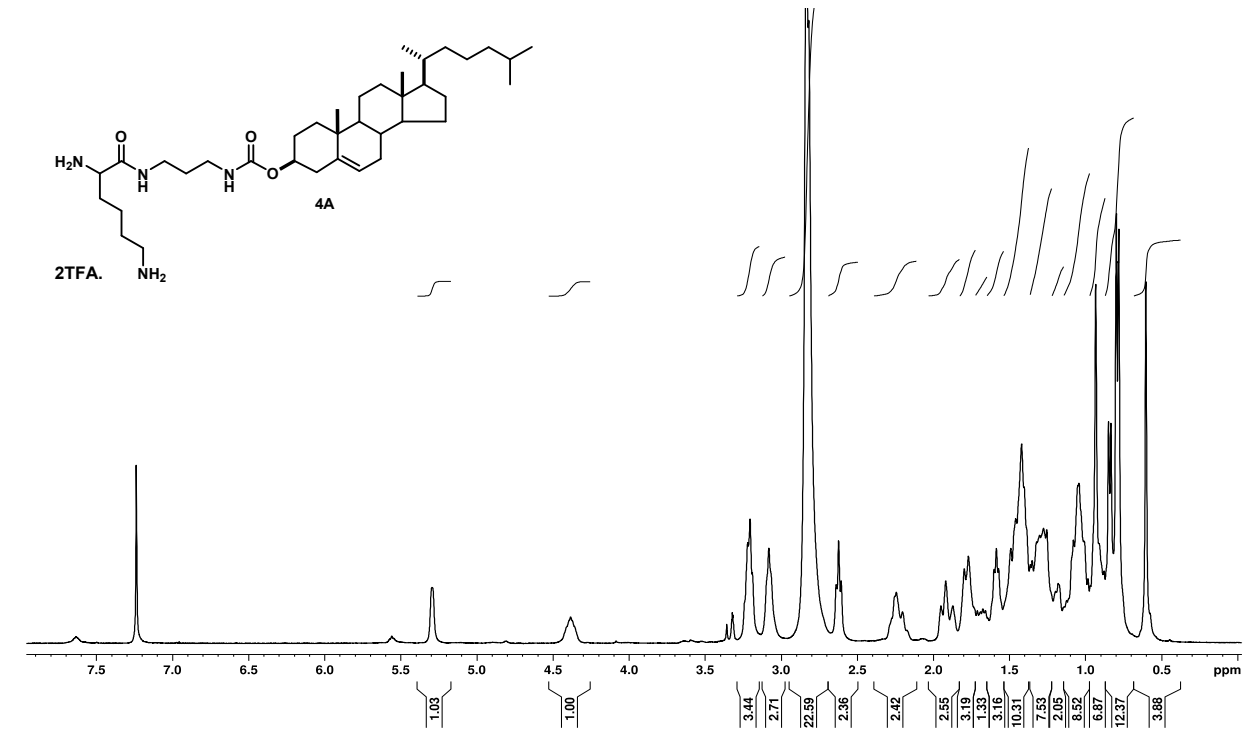
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



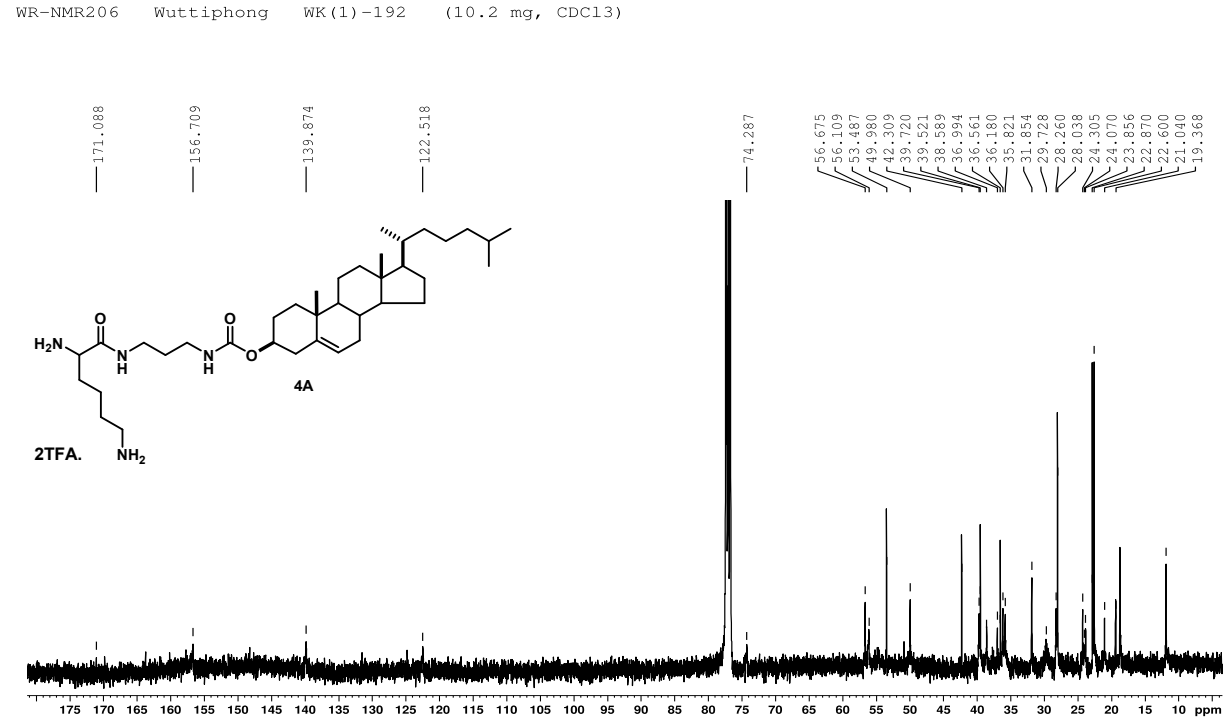
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I a	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
601.505040	1	C36H65N4O3	601.505118	0.1	0.8	6.5	ok	even	13.7	18.3	1.5	5.8	0.8	842.7
	1	C36H65N4O3	601.505118	0.1	0.8	6.5	ok	even	13.7	18.3	1.5	5.8	0.8	842.7
	1	C34H66N4NaO3	601.502713	3.9	-3.2	3.5	ok	even	4.9	7.0	2.4	3.2	0.8	842.7
	2	C39H66N2NaO	601.506735	-2.8	3.6	7.5	ok	even	26.2	35.1	2.6	10.9	1.0	959.8

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:21:20 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N'*-lysinamide)aminoethyl)carbamoyl]cholesterol (**1A**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-lysinamide)aminopropyl)carbamoyl] cholesterol (**4A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-lysinamide)aminopropyl)carbamoyl] cholesterol (**4A**)

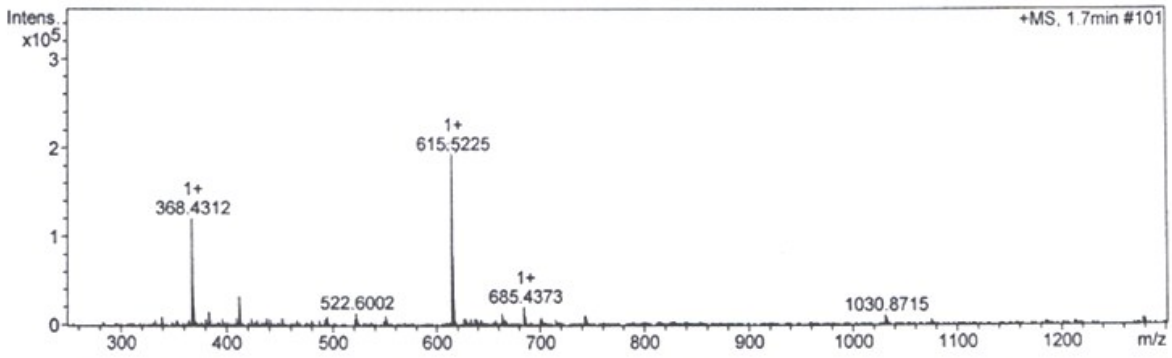
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-149 (pos).d	Acquisition Date	1/21/2016 11:35:57 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK(1)-192/13-14-F1-F13	Instrument	micrOTOF 8213750.10411
Comment			

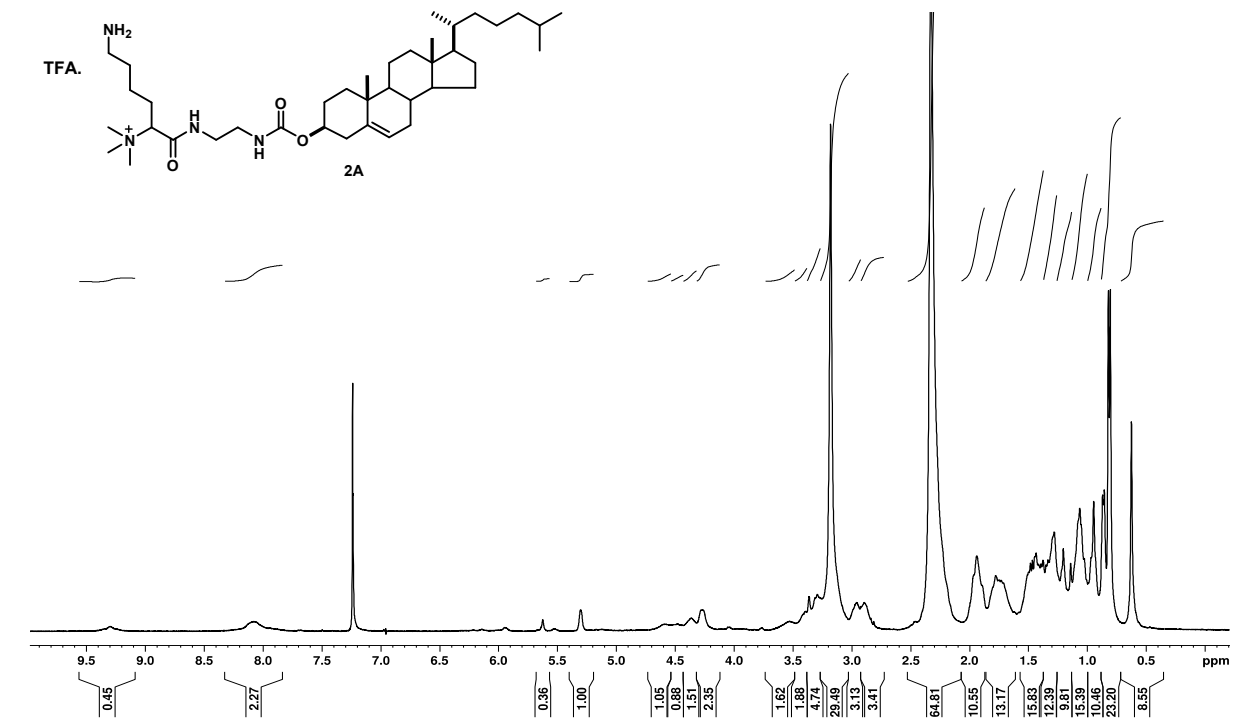
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

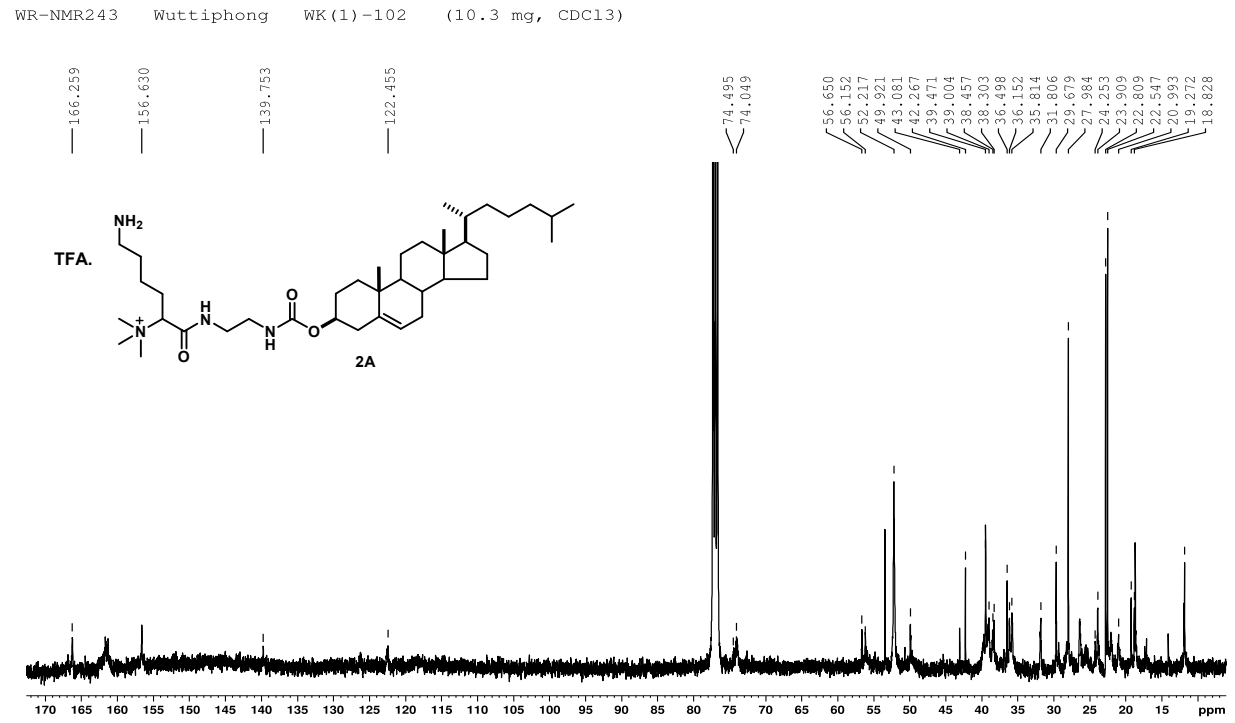


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
615.522527	1	C36H71O7	615.519431	-5.0	-4.9	1.5	ok	even	6.4	10.0	3.2	3.4	0.2	884.0
	2	C37H67N4O3	615.520768	2.9	-2.9	6.5	ok	even	6.7	8.3	2.0	3.6	0.4	842.7
	3	C40H68N2NaO	615.522386	-0.2	-0.2	7.5	ok	even	18.4	22.9	1.1	7.5	0.2	875.6
	4	C42H67N2O	615.524791	-3.7	3.7	10.5	ok	even	29.7	37.9	2.5	11.9	0.2	962.5
	1	C36H71O7	615.519431	-5.0	-4.9	1.5	ok	even	6.4	10.0	3.2	3.4	0.2	884.0
	2	C37H67N4O3	615.520768	2.9	-2.9	6.5	ok	even	6.7	8.3	2.0	3.6	0.4	842.7
	3	C40H68N2NaO	615.522386	-0.2	-0.2	7.5	ok	even	18.4	22.9	1.1	7.5	0.2	875.6
	4	C42H67N2O	615.524791	-3.7	3.7	10.5	ok	even	29.7	37.9	2.5	11.9	0.2	962.5
	1	C38H69N2Na2O	615.519980	4.1	-4.1	4.5	ok	even	8.7	11.1	2.7	4.9	0.2	934.2
	2	C40H68N2NaO	615.522386	-0.2	-0.2	7.5	ok	even	18.4	22.9	1.1	7.5	0.2	887.2

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N'*-lysinamide)aminopropyl)carbamoyl] cholesterol (**4A**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminoethyl)carbamoyl]cholesterol (**2A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminoethyl)carbamoyl]cholesterol (**2A**)

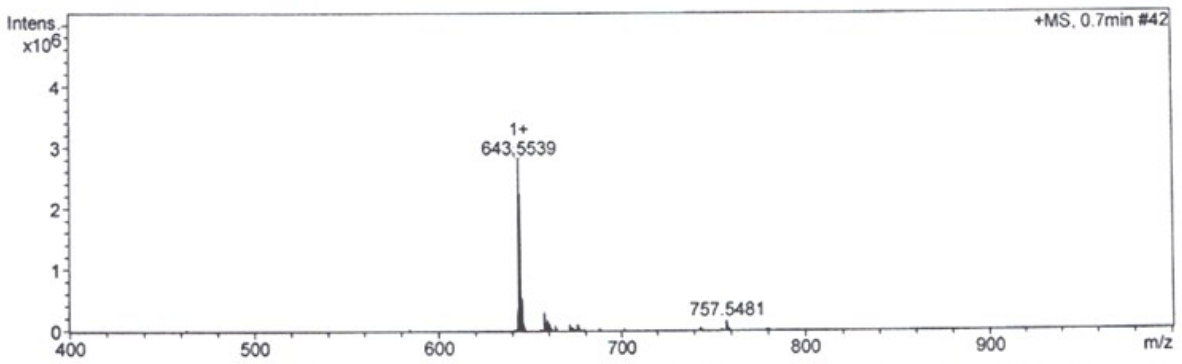
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-143 (pos).d	Acquisition Date	1/21/2016 11:09:49 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-150/9-10	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

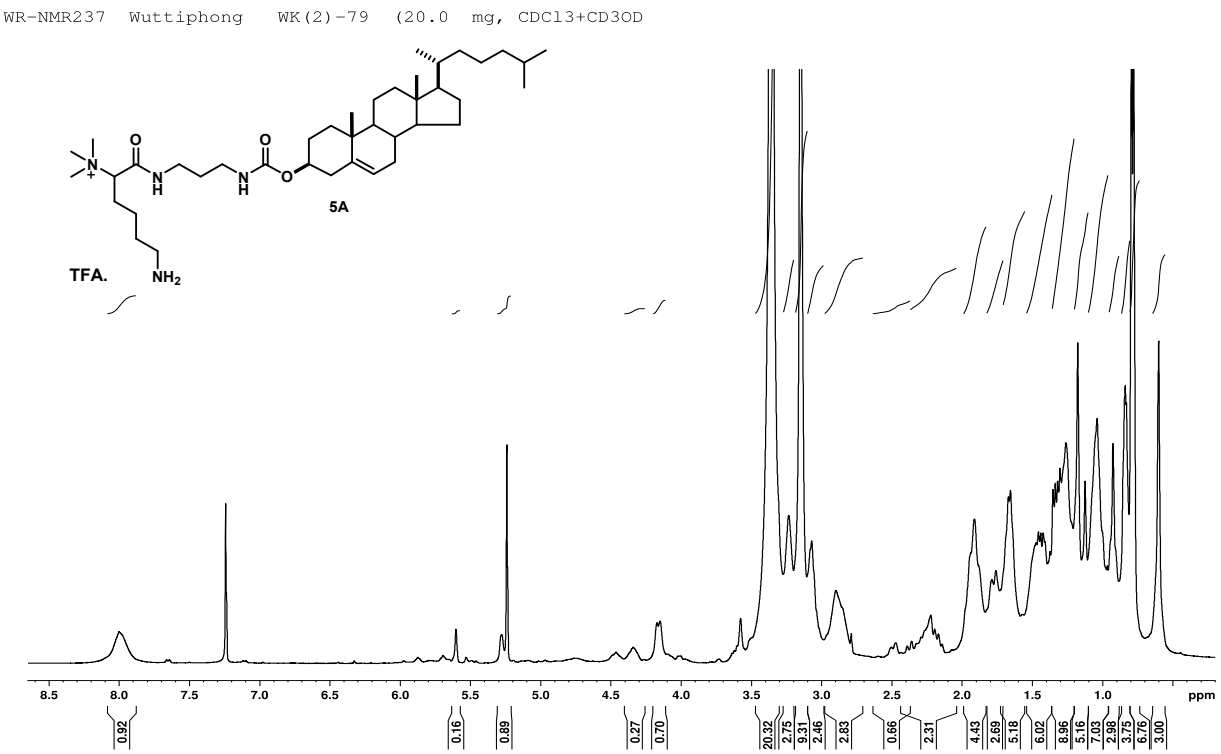
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



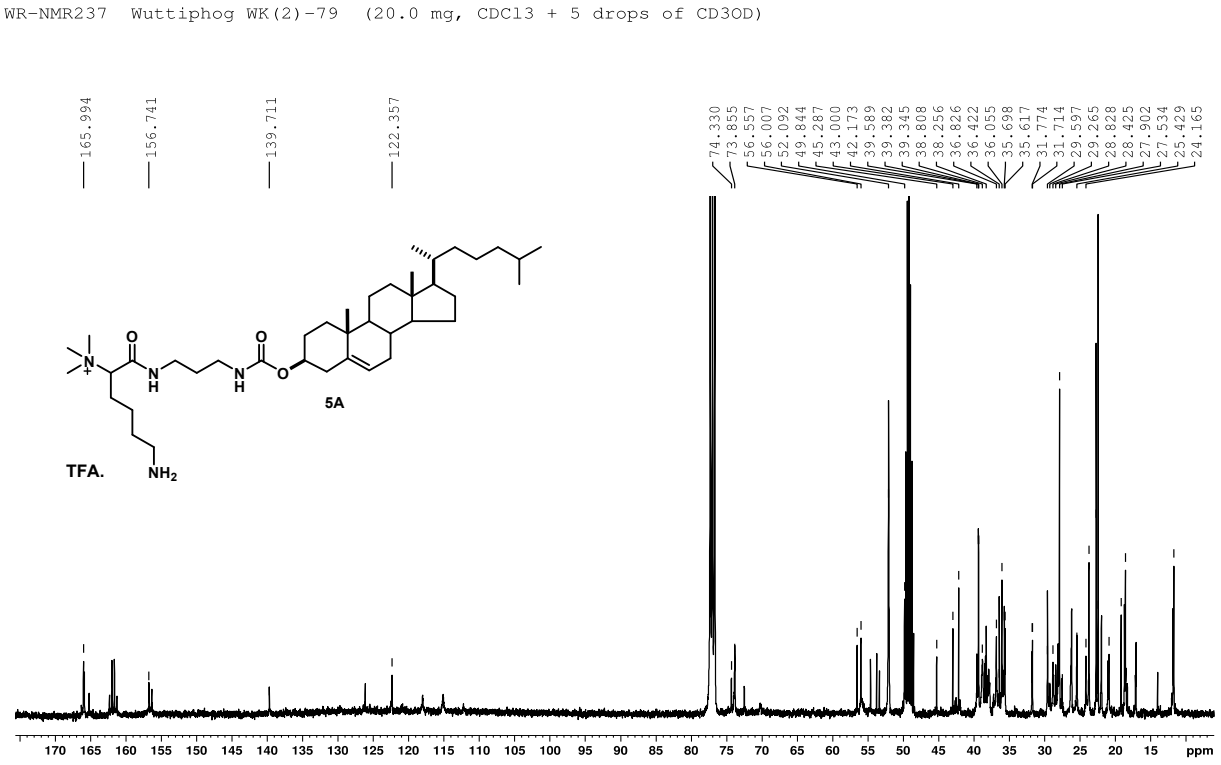
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
643.553874	1	C44H71N2O	643.556091	-3.4	2.9	10.5	ok	even	155.1	150.0	2.0	46.7	1.3	807.5
	2	C39H71N4O3	643.552069	2.8	-3.4	6.5	ok	even	179.5	181.5	2.3	56.6	1.3	842.7
	3	C38H75O7	643.550731	4.9	-5.3	1.5	ok	even	191.1	195.8	3.5	61.3	1.2	891.1
	1	C44H71N2O	643.556091	-3.4	2.9	10.5	ok	even	155.1	150.0	2.0	46.7	1.3	807.5
	2	C39H71N4O3	643.552069	2.8	-3.4	6.5	ok	even	179.5	181.5	2.3	56.6	1.3	842.7
	3	C38H75O7	643.550731	4.9	-5.3	1.5	ok	even	191.1	195.8	3.5	61.3	1.2	891.1
	1	C42H72N2NaO	643.553686	-0.3	-0.8	7.5	ok	even	166.8	165.2	0.9	51.4	1.3	842.7

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:32:43 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N*′-(*N*″,*N*″,*N*″,-trimethylammonium-lysinamide))aminoethyl)carbamoyl]cholesterol (**2A**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminopropyl)carbamoyl]cholesterol (**5A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminopropyl)carbamoyl]cholesterol (**5A**)

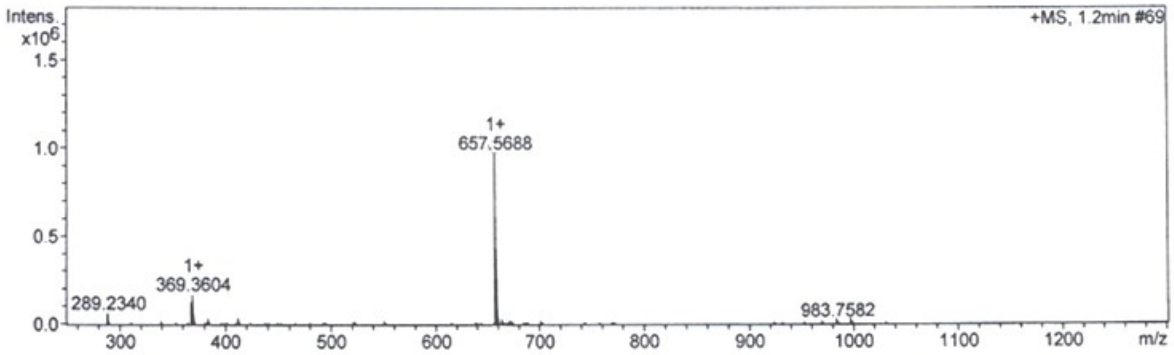
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-151 (pos).d	Acquisition Date	1/21/2016 11:45:05 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (2)-25/11-15	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

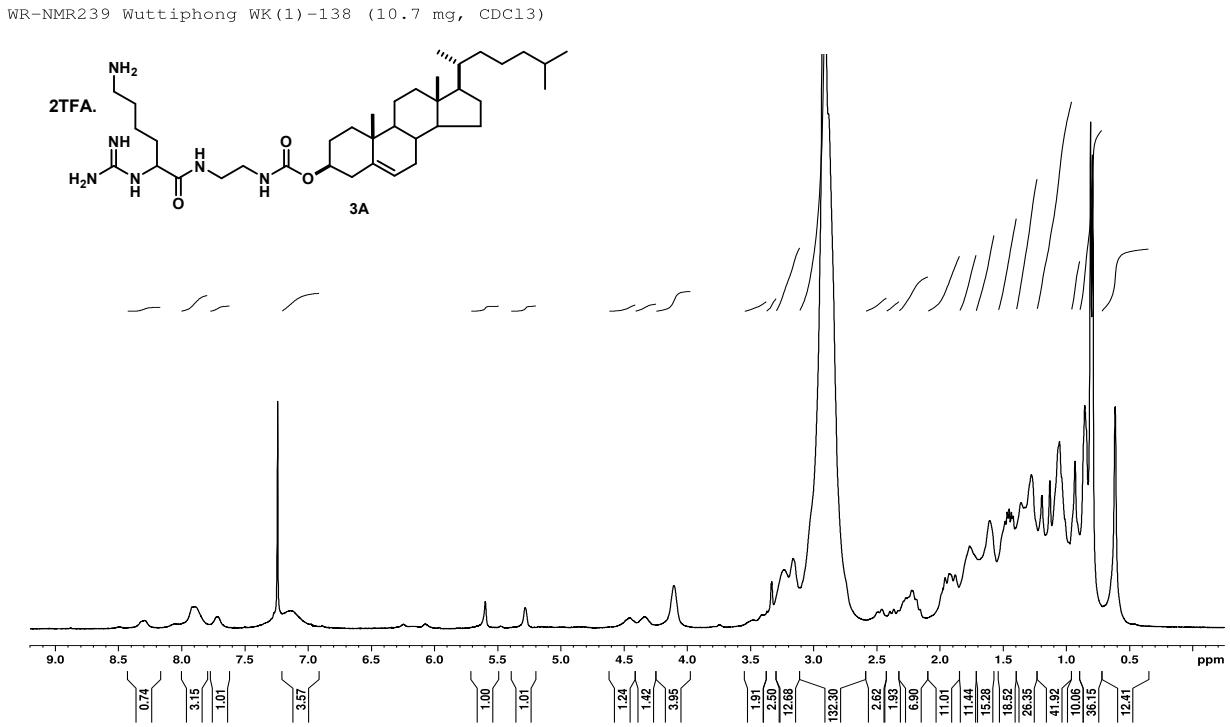
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



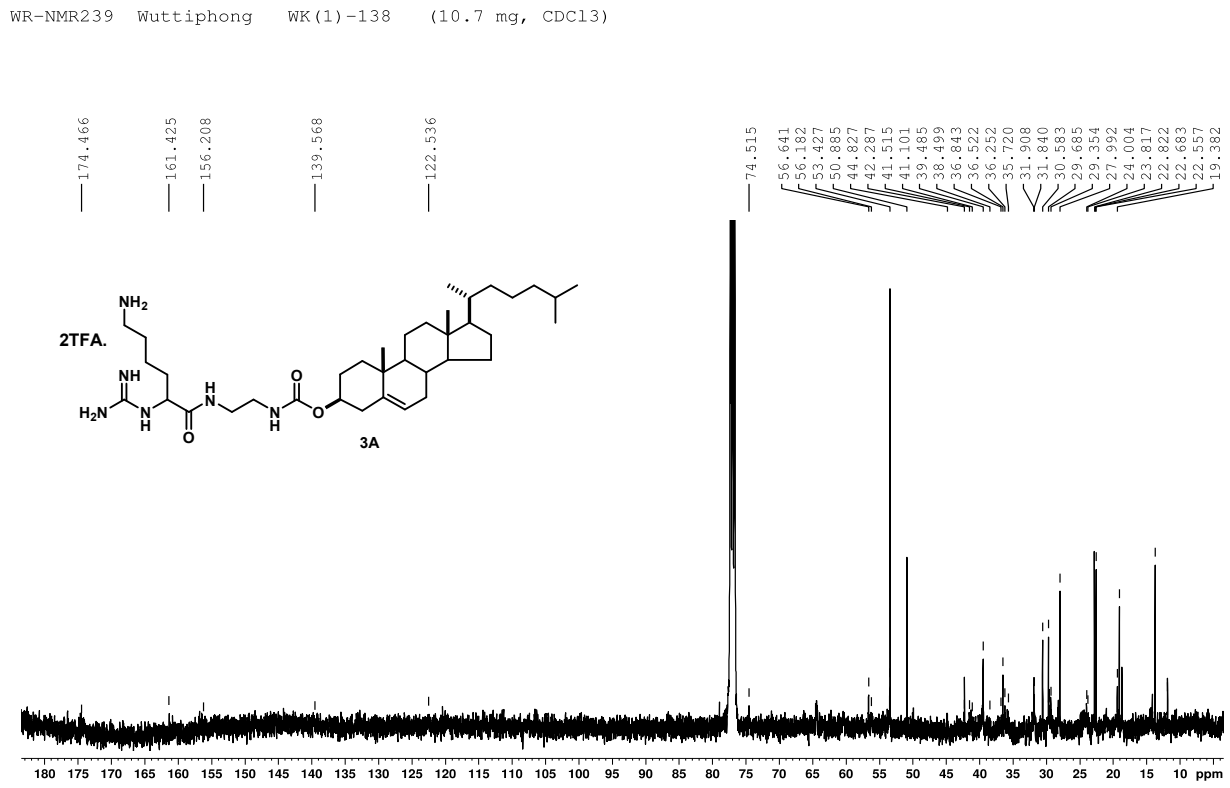
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
657.568832	1	C39H77O7	657.566381	-3.7	-3.1	1.5	ok	even	4.7	5.9	2.2	1.9	0.9	781.5
	2	C38H74N4NaO3	657.565313	-5.4	-4.9	3.5	ok	even	5.8	9.2	3.3	3.4	0.8	882.8
	3	C40H73N4O3	657.567719	-1.7	-1.2	6.5	ok	even	7.1	8.6	1.2	2.7	0.8	747.0
	4	C43H74N2NaO	657.569336	-0.8	1.4	7.5	ok	even	19.7	24.5	1.3	7.6	0.9	889.1
	5	C45H73N2O	657.571741	-4.4	5.0	10.5	ok	even	31.5	39.5	3.4	12.5	1.0	973.9
	1	C39H77O7	657.566381	-3.7	-3.1	1.5	ok	even	4.7	5.9	2.2	1.9	0.9	781.5
	2	C38H74N4NaO3	657.565313	-5.4	-4.9	3.5	ok	even	5.8	9.2	3.3	3.4	0.8	882.8
	3	C40H73N4O3	657.567719	-1.7	-1.2	6.5	ok	even	7.1	8.6	1.2	2.7	0.8	747.0
	4	C43H74N2NaO	657.569336	-0.8	1.4	7.5	ok	even	19.7	24.5	1.3	7.6	0.9	889.1
	5	C45H73N2O	657.571741	-4.4	5.0	10.5	ok	even	31.5	39.5	3.4	12.5	1.0	973.9
	1	C38H74N4NaO3	657.565313	-5.4	-4.9	3.5	ok	even	5.8	9.2	3.3	3.4	0.8	902.7
	2	C41H75N2Na2O	657.566930	-2.9	-2.3	4.5	ok	even	8.6	10.2	1.8	3.5	0.9	842.7
	3	C43H74N2NaO	657.569336	-0.8	1.4	7.5	ok	even	19.7	24.5	1.3	7.6	0.9	899.3

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:47:50 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N′*-(*N″*,*N″*,*N″*,-trimethylammonium-lysinamide))aminopropyl)carbamoyl]cholesterol (**5A**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*-guanidinyl-lysinamide))aminoethyl)carbamoyl]cholesterol (**3A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(2-(*N′*-(*N″*-guanidinyl-lysinamide))aminoethyl)carbamoyl]cholesterol (**3A**)

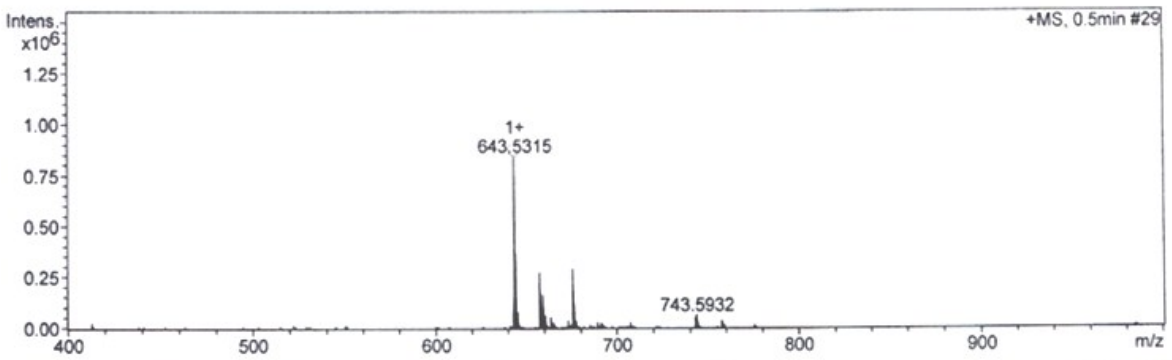
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS-144 (pos).d	Acquisition Date	1/21/2016 11:13:04 AM
Method	tune_wide.m	Operator	RU
Sample Name	WK (1)-140/10-12	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

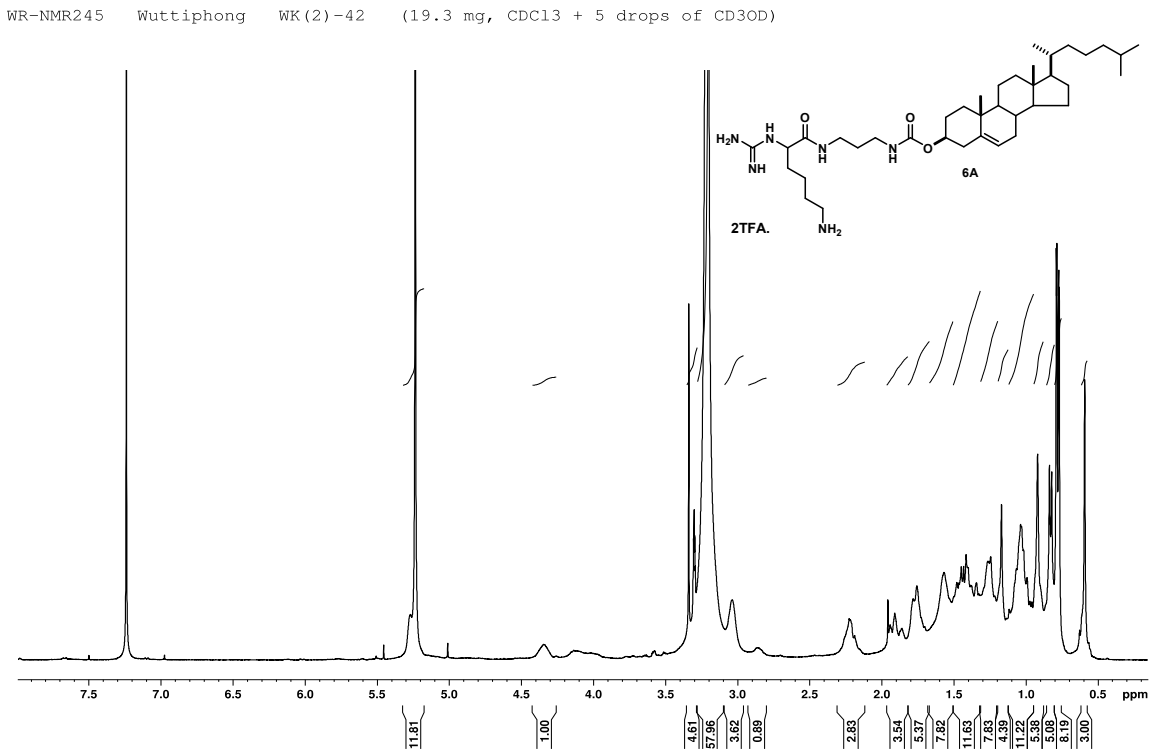
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



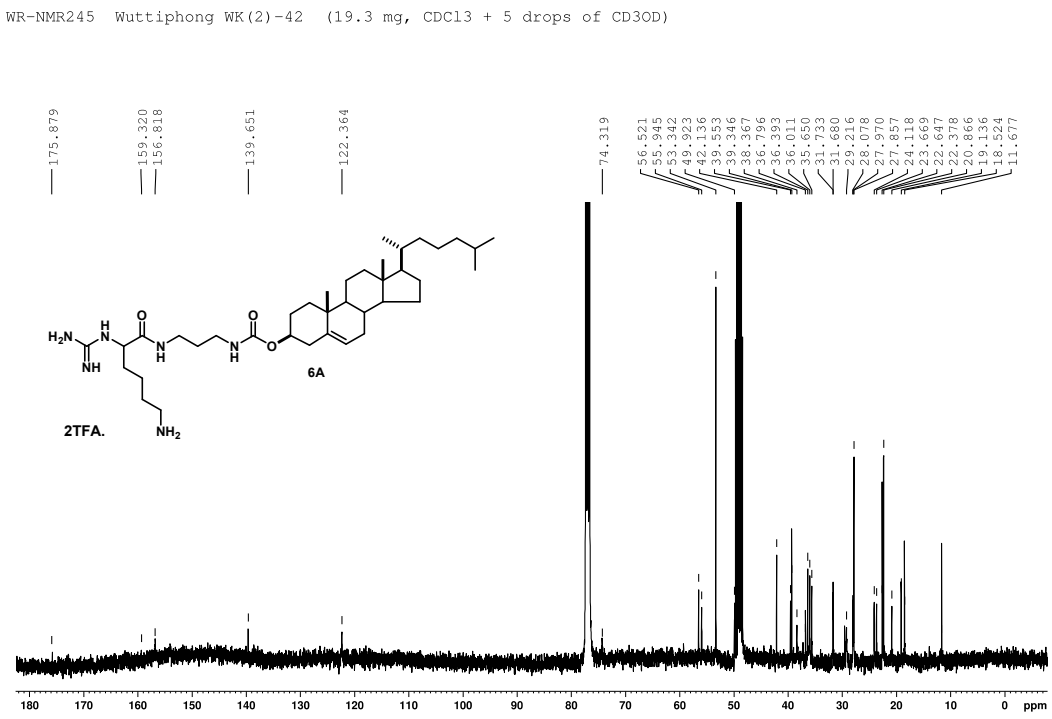
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
643.531525	1	C37H67N6O3	643.526916	-7.2	-6.8	7.5	ok	even	4.6	6.4	4.4	2.1	0.9	692.5
	2	C40H71N2O4	643.540835	-14.5	15.0	6.5	ok	even	6.2	9.1	9.7	3.1	1.1	842.1
	3	C41H71O5	643.529602	-3.0	-2.4	6.5	ok	even	9.0	13.3	1.6	4.6	1.2	668.9
	4	C36H71N2O7	643.525579	-9.2	-8.7	2.5	ok	even	16.1	21.1	5.6	6.6	1.0	843.4
	5	C35H71N4O6	643.536812	8.2	8.7	2.5	ok	even	18.4	24.8	5.6	7.8	0.9	859.2
	6	C42H67N4O	643.530939	-0.9	-0.4	11.5	ok	even	19.9	26.0	0.6	8.2	1.1	690.2
	7	C48H67	643.523729	-12.1	-11.5	15.5	ok	even	47.4	60.4	7.4	19.6	1.4	957.8
	1	C37H67N6O3	643.526916	-7.2	-6.8	7.5	ok	even	4.6	6.4	4.4	2.1	0.9	692.5
	2	C40H71N2O4	643.540835	-14.5	15.0	6.5	ok	even	6.2	9.1	9.7	3.1	1.1	842.1
	3	C41H71O5	643.529602	-3.0	-2.4	6.5	ok	even	9.0	13.3	1.6	4.6	1.2	668.9
	4	C36H71N2O7	643.525579	-9.2	-8.7	2.5	ok	even	16.1	21.1	5.6	6.6	1.0	843.4
	5	C35H71N4O6	643.536812	8.2	8.7	2.5	ok	even	18.4	24.8	5.6	7.8	0.9	859.2
	6	C42H67N4O	643.530939	-0.9	-0.4	11.5	ok	even	19.9	26.0	0.6	8.2	1.1	690.2
	7	C48H67	643.523729	-12.1	-11.5	15.5	ok	even	47.4	60.4	7.4	19.6	1.4	957.8
	1	C39H72NaO5	643.527196	-6.7	-6.1	3.5	ok	even	3.8	4.6	4.0	1.6	1.2	699.0
	2	C38H72N2NaO4	643.538429	10.7	11.3	3.5	ok	even	5.8	7.7	7.3	2.4	1.1	818.5
	3	C39H68N6Na	643.539767	-12.8	13.2	8.5	ok	even	6.1	7.4	8.5	2.6	0.9	844.2
	4	C40H68N4NaO	643.528534	4.6	-4.2	8.5	ok	even	8.2	10.4	2.7	3.3	1.1	752.0
	5	C35H68N6NaO3	643.524511	10.9	-10.5	4.5	ok	even	16.2	23.3	6.8	7.4	0.8	923.4
	6	C34H72N2NaO7	643.523173	-13.0	-12.5	-0.5	ok	even	27.5	37.9	8.0	11.9	1.0	965.4
	7	C33H72N4NaO6	643.534407	4.5	4.9	-0.5	ok	even	29.9	42.0	3.2	13.2	0.9	942.7
	1	C40H71N2O4	643.540835	-14.5	15.0	6.5	ok	even	6.2	9.1	9.7	3.1	1.1	842.1
	2	C48H67	643.523729	-12.1	-11.5	15.5	ok	even	47.4	60.4	7.4	19.6	1.4	957.8
	1	C38H72N2NaO4	643.538429	10.7	11.3	3.5	ok	even	5.8	7.7	7.3	2.4	1.1	818.5

Bruker Compass DataAnalysis 4.1 printed: 1/21/2016 2:34:18 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(2-(*N′*-(*N″*-guanidinyl-lysinamide))aminoethyl)carbamoyl] cholesterol (**3A**)



^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*-guanidinyl-lysinamide))aminopropyl)carbamoyl]cholesterol (**6A**)



^{13}C NMR Spectrum (100 MHz, $CDCl_3$) of 3β-[*N*-(3-(*N′*-(*N″*-guanidinyl-lysinamide))aminopropyl)carbamoyl]cholesterol (**6A**)

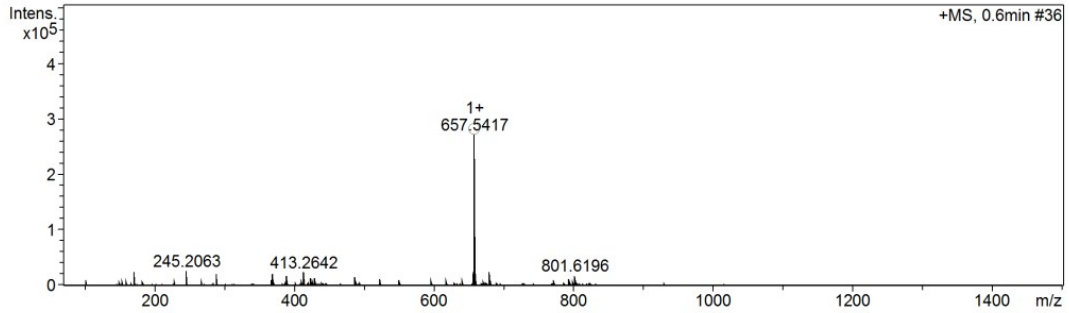
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS 658 (pos).d	Acquisition Date	11/19/2018 3:20:34 PM
Method	tune_low_test.m	Operator	RU
Sample Name	PK(1)-5	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	|err| [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma
657.541701	1	C38H69N6O3	657.542567	0.9	-1.3	1.7	7.5	ok	even	13.7
	2	C42H73O5	657.545252	3.6	-5.4	5.9	6.5	ok	even	27.0
	3	C49H69	657.539379	2.3	-3.5	-3.0	15.5	ok	even	65.9

Bruker Compass DataAnalysis 4.1 printed: 11/20/2018 12:33:30 PM by: RU 1 of 1

HR-ESI-MS Spectrum of 3β-[*N*-(3-(*N′*-(*N″*-guanidinyl-lysinamide))aminopropyl) carbamoyl]cholesterol (**6A**)

Purification of active lipid 3β-[*N*-(3-(*N'*-lysinamide)aminopropyl)carbamoyl]cholesterol (4A)

The lipid **4A** for HPLC-HRMS was prepared at 1 µg/µL in methanol : water (1:1). The solution of lipid **4A** (5 µL) was injected onto the reverse phase C18 column (Acclaim^{TM}120: C18 3 µm 120 Å, 2.1x150 mm). ACN : water was used as a mobile phase with a constant flow rate of 0.3 mL/min. The column temperature was set at 40 °C. The total run time was 30 min. The percentage of purification was found at ~93%. (See chromatogram)

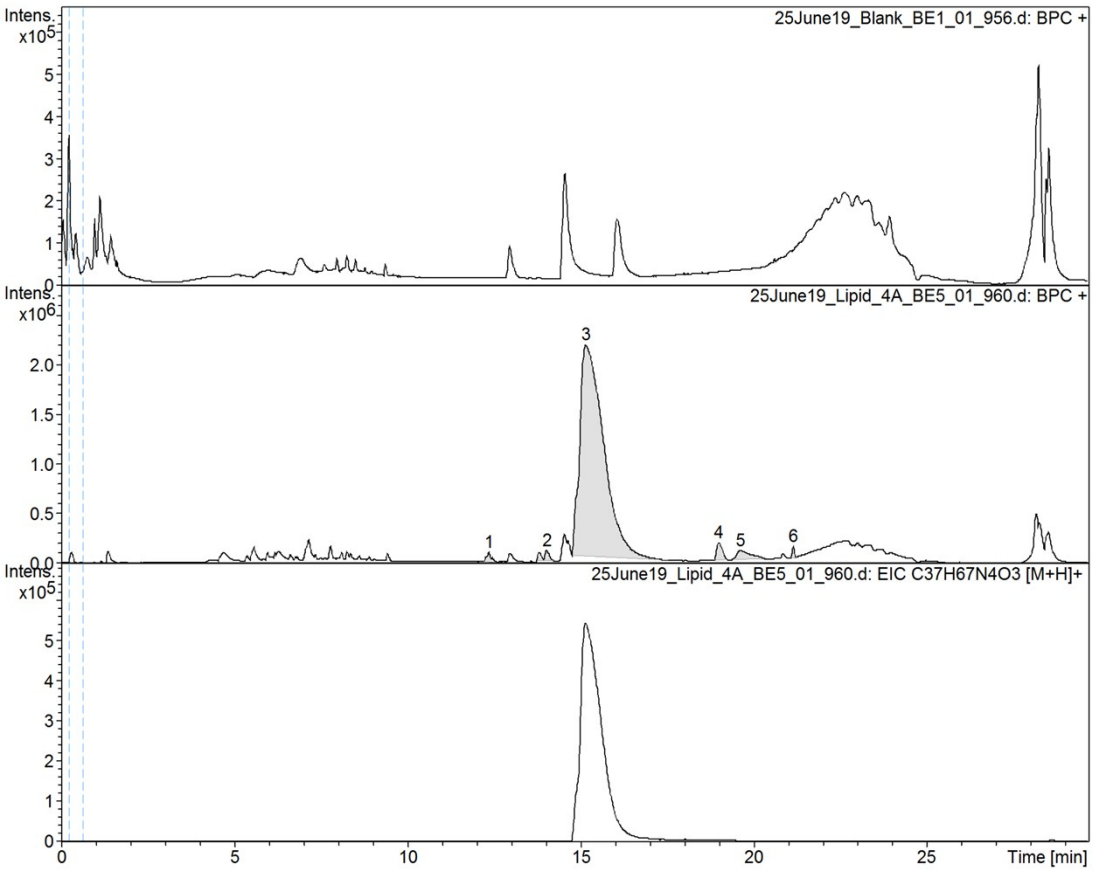
Table S1: Solvent systems

Time	ACN	H_2O
15 min	5%	95%
20 min	100%	0%
25 min	100%	0%
25 min	5%	95%
30 min	5%	95%

$$\% \text{ purity of active lipid } \mathbf{4A} = \frac{102182984}{940775 + 1530827 + 102182984 + 2022050 + 2622850 + 589047} \times 100$$

$$= 92.99\%$$

Generic Display Report (all)



#	RT [min]	Area	Int. Type	I	S/N	Chromatogram	Max. m/z
1	12.3	940775	Manual	111675	67.3	BPC +	274.2756
2	14.0	1530827	Manual	130887	72.7	BPC +	367.3361
3	15.1	102182984	Manual	2195943	1460.8	BPC +	369.3543
4	19.0	2022050	Manual	210458	121.4	BPC +	643.5146
5	19.6	2622850	Manual	130430	62.5	BPC +	487.4250
6	21.1	589047	Manual	170530	79.4	BPC +	282.2801

RU

HPLC-HRMS chromatogram of active lipid **4A**